Henry B. Morison

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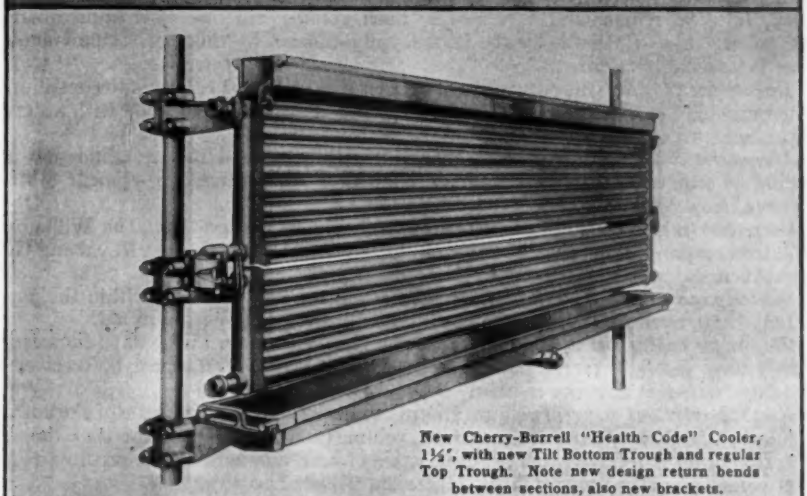

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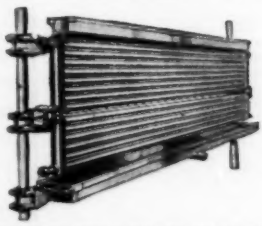

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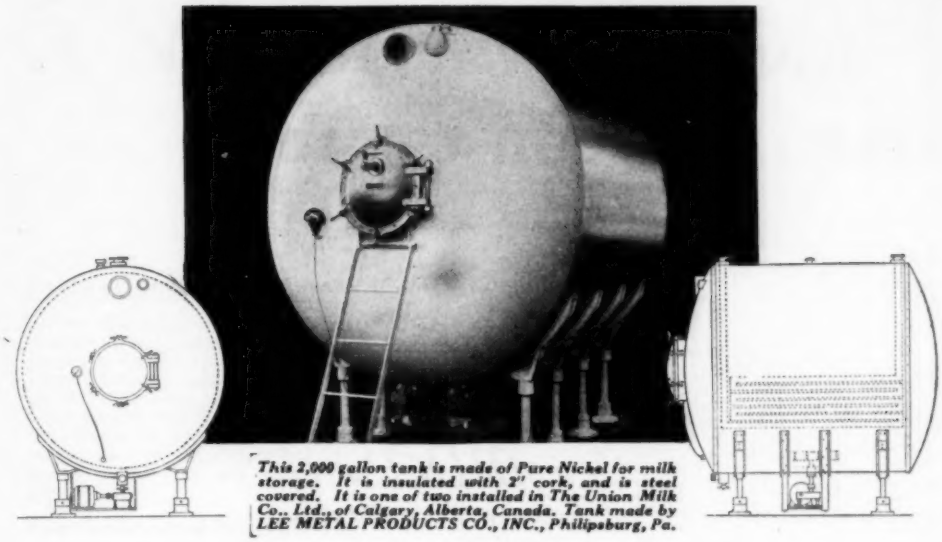

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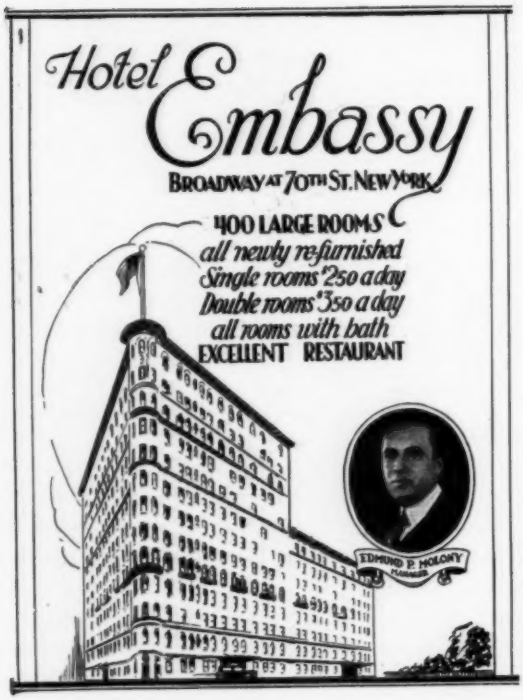

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RELATION BETWEEN TITRATABLE ACIDITY AND HYDROGEN ION CONCENTRATION OF ICE CREAM MIXES*

C. D. DAHLE, W. K. BUDGE AND J. I. KEITH†

Department of Dairy Husbandry, Pennsylvania State College, State College, Pa.

It has long been known that the titratable acidity of milk freshly drawn from the cow does not indicate the true acidity, and that the acidity as it then appears, when determined by means of the ordinary acid tests, is merely apparent acidity. Van Slyke and Bosworth (1) showed that this acidity is due to the presence of substances other than lactic acid in the milk. McInerney (2) noted that milk freshly drawn from the cow gave an acid reaction to titration with an alkali which he calls apparent acidity due to carbon dioxide, acid phosphates and casein. The apparent acidity was found to range between 0.10 and 0.22 per cent and milks high in milk solids-not-fat were found to be higher in apparent acidity than milks of lower milk solids-not-fat content. Rice and Markely (3) found the acidity of cow's milk, calculated as lactic acid, to vary from 0.086 to 0.229 per cent and the hydrogen ion content to vary from pH 6.3 to 7.2, with an average of pH 6.5.

Sharp and McInerney (4) obtained samples of milk which ranged in acidity from 0.05 to 0.50 per cent, calculated as lactic acid and in hydrogen ion concentration from pH 6.0 to 7.73. By plotting the titratable acidity of the samples against the pH values, they found that a definite relation existed, but that the relationship for fresh milk was different from that after lactic acid had formed in the milk due to the action of bacteria.

* Received for publication March 24, 1930. Publication authorized by the Director of Pennsylvania State College Agricultural Experiment Station as Technical Paper No. 500.

† Part of the material presented in this paper forms a part of the thesis of W. K. Budge, submitted in partial fulfillment of the requirements for the degree of Master of Science, in the Graduate School of the Pensylvania State College, 1928.

The relation of the titratable acidity to the hydrogen ion concentration of ice cream mixes has been given little attention by investigators. Workers have studied one or the other but have not attempted to show their relation. Turnbow (5) studied the hydrogen ion concentration of 100 mixes and found it to vary between pH 6.2 and 6.35.

Knowledge of any relationship between titratable acidity and hydrogen ion concentration would be welcomed by ice cream production men. Many of these men make a practice of standardizing the acidity of any mixes that exceed a certain standard, as determined by the ordinary acid test. The acid content is reduced to a definite acidity, the point to which it is reduced being established more or less arbitrarily by the plant operators. It is possible to find mixes which are quite high in titratable acidity, but which are low in actual acidity. In a case like this any attempts to standardize the acidity by usual plant methods might result in producing an alkaline mix.

EXPERIMENTAL

Procedure

The mixes studied in this experiment were made for the most part from cream, condensed skimmilk, skimmilk, sugar, and gelatin. Mixes of several distinct compositions were used, but all were normal commercial mixes in every respect. All mixes were pasteurized at 145°F. for thirty minutes and homogenized at a pressure of 2500 pounds at the pasteurizing temperature, cooled to 40°F., aged for twenty-four hours and the determinations made.

The titratable acidity calculated as lactic acid was determined on 9 grams of mix, diluted with 9 grams of distilled water. This diluted mix was titrated with N/10 sodium hydroxide, using phenolphthalein as an indicator. The hydrogen ion concentration was determined electrometrically at 25°C. The quinhydrone electrode was used. Lester (6) determined the hydrogen ion concentration of 10 samples of whole milk, cream and whey with the quinhydrone electrode and also the bubbling hydrogen elec-

trode. The pH values of the two methods varied, on the average, less than 0.05 pH.

The acidity and pH of normal mixes

The data presented in figures 1, 2 and 3 were obtained from normal ice cream mixes made according to the method just described. These mixes contained 12 per cent fat but the serum solid content varied from 10 per cent to 12 per cent. The data are assembled according to serum solid content. Mixes listed

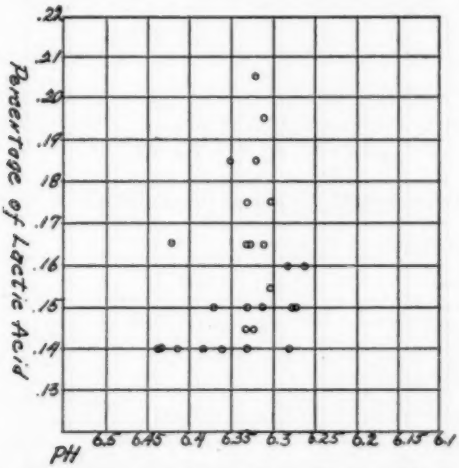

FIG. 1. TITRATABLE ACIDITY AND pH OF MIXES TESTING 10 PER CENT SERUM SOLIDS

under 10 and 12 per cent serum solids were made from cream, condensed skimmilk and skimmilk, while some of those listed under 11.7 per cent serum solids contained some unsalted butter and dry skimmilk of various types. The majority of hydrogen ion determinations fell in the range pH 6.25 to 6.35, with an average fairly close to pH 6.3.

The titratable acidity was highest in the mixes containing the largest percentage of serum solids. This has been demonstrated before (7) and this knowledge is of importance to the ice cream

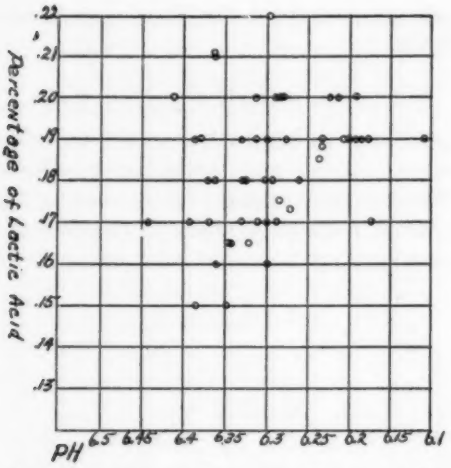

FIG. 2. TITRATABLE ACIDITY AND pH OF MIXES TESTING 11.7 PER CENT SERUM SOLIDS

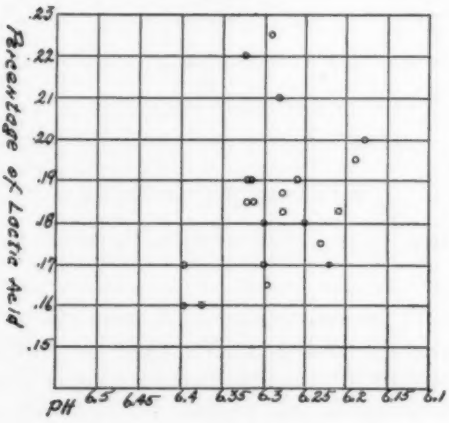

FIG. 3. TITRATABLE ACIDITY AND pH OF MIXES TESTING 12 PER CENT SERUM SOLIDS

maker, particularly if he standardizes the acidity of the mix in the manufacturing process. The hydrogen ion concentration also

was greatest in the mixes of higher serum solid concentration. Table 1 shows the average titratable acidity and hydrogen ion concentration for the mixes used.

That no direct relationship exists between titratable acidity and hydrogen ion concentration may be seen from figures 1, 2 and 3, particularly figure 2. In this collection may be found several mixes having a titratable acidity of 0.19 per cent but ranging in hydrogen ion concentration from pH 6.119 to 6.41. At the same time, mixes were found ranging in acidity from 0.15 to 0.21 per cent and having a hydrogen ion concentration of pH 6.3.

The acidity and pH of mixes permitted to develop acid

To study further the relation of titratable acidity and hydrogen ion concentration, several mixes of definite composition were made

TABLE 1

The effect of serum solids on the titratable acidity and pH of mixes

	PERCENTAGE OF SERUM SOLIDS					
	10		11.7		12	
	pH	Acidity	pH	Acidity	pH	Acidity
Highest	6.42	0.205	6.411	0.21	6.389	0.225
Lowest	6.16	0.140	6.119	0.15	6.178	0.16
Average	6.323	0.1593	6.294	0.1832	6.282	0.1851

and a small amount of lactic culture added after cooling them to 70°F. At intervals, acidity and pH determinations were made. Three mixes for each composition were studied; the results of these studies are shown in figure 4, lines A, B and C. A more direct relationship existed between the pH and titratable acidity in the mixes containing developed acid. While no definite curve could be drawn for the normal mixes, it was found possible to plot a curve with the latter. It is natural to expect that after the acid has begun to develop, a definite curve could be plotted, as measurements at this time indicate true acidity. These curves do not coincide with the curve that Sharp and McInerney obtained with fresh and sour milk. This would not be expected

since the initial acidities of mixes may differ widely from those of samples of milks. This is due to the difference in composition of milk and ice cream mixes.

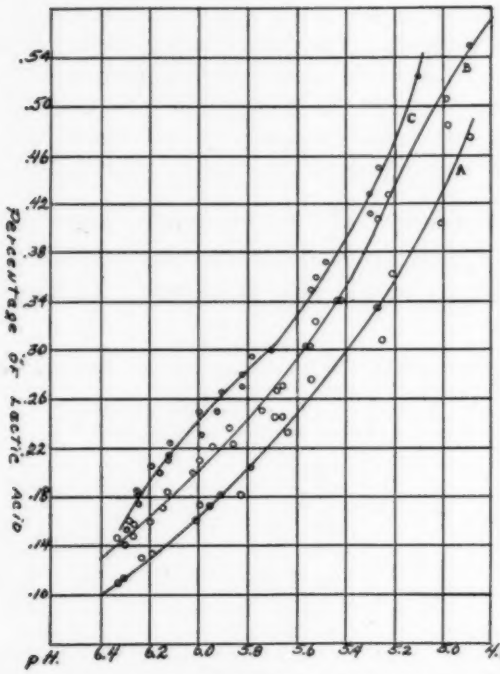

FIG. 4. TITRATABLE ACIDITY AND pH OF MIXES THAT HAVE BEEN PERMITTED TO SOUR

A. Mix containing 8 per cent serum solids.
B. Mix containing 10 per cent serum solids.
C. Mix containing 12 per cent serum solids.

SUMMARY

The titratable acidity of a mix is not a true indication of the amount of lactic acid present.

The figures obtained for titratable acidity are meaningless unless the serum solid content is known.

Knowledge of the serum solid content of a mix, and of its titratable acidity, are not sufficient to enable one to judge the correct point to which the acid may be standardized.

REFERENCES

(1) VAN SLYKE, L. L., AND BOSWORTH, A. W. 1914 The cause of acidity of fresh milk of cows and a method for determination of acidity. N. Y. Agri. Exp. Sta. Tech. Bul. 37.

(2) MCINERNEY, T. J. 1924 A note on the acidity of fresh milk. Jour. Dairy Sci., v, no. 3, 227.

(3) RICE, F. E., AND MARKLEY, A. L. 1924 The relation of natural acidity in milk to composition and physical properties. Jour. Dairy Sci., vii, no. 5, 468.

(4) SHARP, P. F., AND MCINERNEY, T. J. 1927 The relation of the hydrogen ion concentration to titratable acidity of milk. Jour. Biol. Chem., lxxv, no. 1, 177.

(5) TURNBOW, G. D. 1927 Can density be controlled? Ice Cream Trade Jour., xxiii, no. 1, 379.

(6) LESTER, VEDA. 1924 On the measurement of hydrogen ion concentration in some dairy products by means of the Biilman quinhydrone electrode. Jour. Agri. Sci., xiv, no. 4, 634.

(7) DAHLE, C. D. 1926 The acidity question. Ice Cream Trade Jour., xxii, no. 8, 46.

EFFECT OF A DIET OF SWEET CLOVER ON THE CALCIUM IN THE BLOOD SERUM*

C. Y. CANNON

Iowa State College†

AND

DELBERT GREENWOOD

Brigham Young University

On account of the splendid value of sweet clover as an aid to soil fertility and also because of its wide adaptability to soil and climatic conditions, its use as a cattle feed has been expanding rather rapidly during the last few years. A demand thus arose to know its feeding value. As a result of this demand some trials were run to compare it with alfalfa and other hays. The majority of the trials point to the conclusion that good sweet clover hay equals alfalfa hay as a source of protein in the dairy cow's ration.

Notwithstanding the high value of sweet clover hay there is a serious reaction against its use because of the numerous deaths in cattle traceable to its use. The first suggestion that deaths in cattle were occasioned by the use of sweet clover was reported by Schofield (14). Up until this time deaths that may have been caused by sweet clover were attributed to other causes for the symptoms were somewhat confusing.

Later Fitch (2), Mayo (9), Broerman (1), Schalk (13), Graham, McCullock, and Grindley (3), all reported observations where cattle deaths were brought about through the feeding of sweet clover hay. Some of these reports stated that death came because the hay was damaged or moldy. Roderick (11) says "It seems to be definitely limited to sweet clover and only when in the form of spoiled hay or silage."

Probably the most intense study of this disease and its causes is that reported by Schofield (15). His observations led him to the conclusion that this disease was induced by molds that grew on

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† Formerly at Brigham Young University.

the hay. He held that a casual examination would not always disclose the presence of mold, for often times bright appearing hay would show molds in the stems of the plant. He tried feeding rabbits on the hay and found that they responded much as did cattle except that the response was more rapid.

He then attempted to isolate the organism that might be responsible for the reaction in these animals as he is of the opinion that "the theory that the hemorrhage is the result of the delayed clotting time of the blood is not sound," but that a powerful poison is developed which causes degeneration in the walls of blood vessels and capillary endothelium which permits severe ruptures of the blood vessels. Roderick (11) also maintains the theory of toxic injury to the vessel walls.

He also points out that a "prolonged coagulation time and a low percentage of hemoglobin are two conditions frequently observed in connection with this disease whether experimental or natural. The acute form of the disease can occur without either of these changes, but in the sub-acute form it is most likely that both are always present to some extent." He suggests that delayed clotting is "due either to an absence of thrombin or an inhibition of thrombin."

Roderick and Schalk (12) suggest that the best way to tell whether a hay is safe for cattle feeding or not is to feed rabbits on the same hay with the cattle and since the rabbits show the disease symptoms more quickly than do the cattle, one can tell when the hay is unsafe in time to remove the cattle from the feed so as to avoid serious trouble.

As far as we can find no work has been reported which indicates what has happened in the blood of animals which were fed sweet clover hay or silage to cause the blood to lose its power of coagulation. Most writers made suggestions of interference in thrombin formation.

The question arose with us as to whether any interference with the serum calcium had been brought about by the sweet clover ration. As rabbits had been observed by Schofield (15), and Roderick and Schalk (12) to show so clearly the symptoms we wished to study, it was decided to use them as subjects for this

observation in order to determine what changes, if any, take place in their blood-serum calcium while on different diets.

EXPERIMENTAL

The sweet clover (Melilotus alba) used in this trial was gathered during the summer of 1928. It was taken from a heavy stand, growing on a clay soil which was rather rich in alkali.

This feed was gathered by hand with only the finer branches, thickly covered with leaves being taken. These samples were immediately spread on large canvass sheets and exposed to the hot summer sun. The humidity of the air was low which insured a rapid curing. At night the hay was covered by canvass to protect it from any dew. This hay was stored in bags hung in a basement near a furnace so that the temperature of exposure ranged from about 50° to 80°F. Just before the feeding trial this hay was ground to a rather coarse meal.

Three lots of rabbits about three months of age were used in this experiment.

Lot 1 received a diet of sweet clover meal and distilled water.

Lot 2 was placed on a diet of alfalfa meal and distilled water.

Lot 3 received a balanced diet consisting of alfalfa meal, oats, and fresh cabbage or lettuce daily plus water.

Calcium determinations of the serum and weighings of each rabbit were made each week for a period of about two months.

Our method of obtaining the blood from the rabbits was to make a heart puncture with a hypodermic needle attached to a Luer syringe. About 5 cc. of blood were extracted at each time. The blood was allowed to coagulate and was then centrifuged. Two cubic centimeters of the colorless blood-serum were analyzed for calcium by the Clark-Collip Modification of the Kramer-Tisdall Method (5). Calcium was precipitated directly from the serum as an oxalate and titrated with N/100 $KMnO_4$. (See table 1.)

DISCUSSION

As we had hypothesized the serum calcium of the rabbits on sweet clover fell sharply from the amount carried at the beginning of the trial. It also dropped as compared with the rabbits on

alfalfa and on alfalfa with supplements. This variation downward was from 16 mgm. or above to as low as 12.8 mgm.

The record of rabbit B was inserted because of its interest. It had reached a low point in its serum calcium much faster than other rabbits on the same diet. In an attempt to take the last blood sample, difficulty with the syringe forced a withdrawal of the needle before enough blood for the analysis was obtained. The

TABLE 1

Typical changes of body weight and blood serum calcium of certain of our rabbits

DATE OF BLOOD SAMPLING	DIET							
	Sweet clover meal				Alfalfa meal		Alfalfa meal, oats, cabbage leaves, etc.	
	Rabbit A		Rabbit B		Rabbit C		Rabbit D	
	Weight	Ca per 100 cc. serum	Weight	Ca per 100 cc. serum	Weight	Ca per 100 cc. serum	Weight	Ca per 100 cc. serum
1919	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*	*grams*	*mgm.*
September 11	1,751	16.8	1,688	16.0	1,675	15.9	1,675	15.9
September 18	1,542	16.0	1,632	15.8	1,678	15.1	1,768	15.0
October 3	1,633	13.2	1,724	14.4	1,905	15.8	2,175	15.4
October 10	1,769	13.0	1,859	12.8	1,996	17.7	2,313	16.4
October 17	1,769	12.8	1,814	†	2,040	16.0	2,358	15.8
October 24	1,905	13.6			1,950	15.6	2,493	16.6
October 31	1,950	12.8			1,950	16.0		‡
November 7	1,950	13.0*			2,086	16.0		
November 14	2,041	15.2			2,086	16.0		
November 21	2,041	15.6			2,086	16.2		
December 6		16.1			1,905	16.2		

* Diet of rabbit changed from sweet clover to alfalfa.

† Rabbit bled to death due to slow coagulation before a complete sample could be obtained.

‡ Accidently broke its neck while sample of blood was being taken.

delay was such that before a new attempt at getting more blood could be made, the rabbit showed signs of labored breathing and other trouble. Within ten minutes it was dead.

An autopsy was immediately made. The thoracic cavity was found filled with blood which showed a definitely delayed power of coagulation. We had anticipated that this result would come providing the sweet clover had its usual effect.

Unfortunately, about the time the remainder of the rabbits reached approximately the same level of serum calcium in their blood, our supply of sweet clover had all been used. The rabbits were then changed from sweet clover meal to alfalfa meal with a resultant quick rise in the serum calcium.

At no time did the calcium in the serum of the rabbits on alfalfa meal or alfalfa meal supplemented with other feeds show any tendency to decline in quantity. Evidently this drop in calcium values in this experiment came only under the influence of feeding sweet clover.

An analysis for calcium of the alfalfa and sweet clover used in this trial showed that the alfalfa contained nearly double the amount of calcium that was contained in the sweet clover.

Alfalfa, besides having a large quantity of calcium in its make-up also seems to have a great power in aiding calcium retention by the body as pointed out by Hart and his co-workers (4).

When sweet clover was fed to the rabbits much less calcium was absorbed by their bodies for as has been pointed out (10) "studies of the blood have furnished additional information in regard to the absorption of these particular elements (Ca and P), a rise of concentration in the serum being taken as an indication of increased absorption, and a fall of impaired absorption."

The injurious effect of an insufficient absorption of calcium is more noticeable with growing animals than with mature animals. There may be some correlation here with the observations that "sweet clover poisoning" occurs more often and with a higher death rate among calves and immature animals than among those fully matured.

The question immediately arises as to the effect of this lowered calcium content of the blood on the apparent symptoms in sweet clover feeding, even though it may vary downward to a very considerable extent. The commonly accepted theories (6) of blood coagulation all recognize the essentiality of calcium in activating thrombin. Calcium apparently is not essential in changing fibrinogen to fibrin (6, 7).

The theory put forth by some investigators that thrombin formation is interfered with due to the consumption of sweet

clover hay or silage would necessarily raise the question of the calcium rôle in the delayed clotting of the blood.

Loucks and Scott (7) have pointed out that the blood platelets, which are supposedly the source from which most of the prothrombin originates, do not rupture unless the surface tension of the blood is definitely lowered. From their experiment it seems that anticoagulants prevent the lowering of the blood surface tension by their effect on the blood calcium. They showed also that by an addition of $CaCl_2$ they obtained a definite lowering of the surface tension which allowed clotting through the apparent rupturing of the platelets. They say "Evidently the lowered surface tension produced by adding calcium is the causative factor in the bursting of platelets."

Any lowering, then, of the blood calcium would cause a rise in the surface tension thereby delaying the disintegration of the platelets. Under these conditions small hemorrhages would take place first and as the calcium continued to be lowered the clotting would be interfered with to a greater extent, thereby permitting larger hemorrhages. It has also been observed (15) that there is a lowered quantity of both the red blood cells and the blood platelets as the sweet clover feeding continues.

Our experiment points out that there is a decrease amounting to as much as 25 per cent in the serum calcium of rabbits fed sweet clover hay. The hay we used was evidently of excellent quality as evidenced by the way it was cured and stored and also by the slowness of its reaction on the rabbits. Certain factors produced in sweet clover hay under adverse couring condition perhaps accentuate the calcium loss from the blood thereby accelerating the onset of clot failure, but even without any apparent damage to the sweet clover hay some changes were set up in the blood which ultimately could influence the health of the animals.

The fact that young animals are more susceptible to the "poisoning" than older ones is definitely pointed out and it sustains the contention of calcium interference. In the case of rickets, which is a disease involving calcium metabolism, the young are decidedly more susceptible than are the old, as pointed out by McCollum and Simmonds (8). Even though good hay gives no

apparent trouble when fed, yet surely it does cause some changes in the blood which if continued over a long period might ultimately have its influence on the well-being of the animal. An examination of the weight charts shows that all three of the diets used were able to support regular growth.

SUMMARY

1. There was a decrease in the serum calcium of young rabbits fed on an exclusive diet of sweet clover meal plus distilled water, while it remained fairly constant when rabbits were fed alfalfa meal plus distilled water or alfalfa meal-oats-cabbage-lettuce diets. Upon the resumption of an alfalfa diet the serum calcium content of the blood returned to normal.

2. The decline in serum calcium brought about by sweet clover is probably linked with the failure of the blood to clot in "poisoning."

3. It is suggested that the loss of serum calcium in the blood might raise the surface tension of the blood to such a point that the blood platelets would fail to rupture thus interfering with the formation of thrombin.

4. Rabbits fed on the sweet clover increased their weight at a rate corresponding with those on the other diets.

REFERENCES

(1) Broerman, Alvin 1925 Poisoning of cattle by sweet clover hay. Jour. Amer. Vet. Med. Assoc., lxvii, 367–71.

(2) Fitch, C. P. 1923 Disease in cattle caused by feeding sweet clover hay. Amer. Soc. Anim. Prod. Proc., 37–41.

(3) Graham, R., McCullock, E. C., and Grindley, H. S. 1928 No danger of poisoning from clean sweet clover. Annual Rpt. III, Agr. Exp. Sta. 1927–1928, 114–115.

(4) Hart, E. B., Steenbock, H., Hoppert, C. A., and Humphrey, G. C. 1922 The comparative efficiency of dry and green alfalfa in maintaining calcium and phosphorus equilibrium in milking cows. Jour. Biol. Chem., lii, 21–30.

(5) Hawk, P. B., and Bergein, Olaf 1926 Practical Physiological Chemistry, p. 408.

(6) Howell, William H. 1915 A Text Book of Physiology, pp. 452–468.

(7) Loucks, Milo M., and Scott, F. H. 1929 Calcium in the coagulation of blood. Amer. Jour. Physiol., xci, 27–46.

(8) McCollum, E. V., and Simmonds, Nina 1929 The Newer Knowledge of Nutrition, p. 272.

(9) Mayo, N. S. 1924 Sweet clover hay poisoning. Jour. Amer. Vet. Med. Assoc., lxv, 229–230.

(10) Orr, W. J., Holt, L. E., Jr., Wilkins, L., and Boone, F. H. 1924 The relation of calcium and phosphorus in the diet to the absorption of these elements from the intestine. Amer. Jour. Dis. Child., xxviii, 575–581.

(11) Roderick, L. M. 1929 The pathology of sweet clover disease in cattle. Jour. Amer. Vet. Med. Assoc., lxxiv, 314–325.

(12) Roderick, L. M., and Schalk, A. F. 1928 Precautions for feeding spoiled sweet clover hay. North Dakota Agr. Expt. Sta. Cir. 35.

(13) Schalk, A. F. 1926 Cattle disease resulting from eating damaged or spoiled sweet clover hay or silage. North Dakota Agr. Expt. Sta. Cir. 27.

(14) Schofield, F. W. 1922 A brief account of a disease in cattle simulating hemorrhagic septicemia due to the feeding of sweet clover. Canad. Vet. Rec., iii, 74–78.

(15) Schofield, F. W. 1924 Damaged sweet clover. The cause of a new disease in cattle simulating hemorrhagic septicemia and blackleg. Jour. Amer. Vet. Med. Assoc., lxiv, 553–575.

THE CALCIUM AND PHOSPHORUS METABOLISM OF HEAVILY MILKING COWS*

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The experiments reported in this paper are part of a mineral feeding project instituted for the purpose of studying the effects of different calcium and phosphorus levels and different mineral supplements on the growth, health, reproduction and milk production of dairy cattle from six months to five years of age.[1]

The investigations of Forbes and his associates (1) indicated that the calcium and usually the phosphorus balances were negative when milk production exceeded 10 pounds per day. Losses of these elements occurred with liberally milking cows regardless of the supply in the ration. Similar results were reported by Hart, Steenbock, Hoppert, Bethke, and Humphrey (2), Meigs, Blatherwick, and Cary (3), and Meigs, Turner, Harding, and Hartman (4). However, Hart, Steenbock, Hoppert and Humphrey (5) stated that they were able to maintain heavily producing cows in positive calcium balance by feeding alfalfa hay cured under caps, and to reduce losses of calcium and phosphorus in cows receiving timothy by the addition of bone meal. Hart and his associates (6) also maintained positive calcium balances by the addition of marl.

Miller and his associates (7) reported increased calcium retention after supplementing the basal ration with bone meal. At the Vermont Agricultural Experiment Station (8) positive calcium and phosphorus balances were maintained when bone meal and limestone rock were added to the ration of liberally milking cows. Perkins and Monroe (9) secured positive calcium balances with liberally milking cows on a narrow ration.

Our own experience indicated that (*a*) cows could usually be

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[1] Other experiments on this project are reported in Michigan Agricultural Experiment Station Technical Bulletin 105.

maintained in positive calcium and phosphorus balance even when producing large amounts of milk and (*b*) that the needs of the animal determined primarily the amount of calcium and phosphorus that would be utilized. Thus on a low calcium ration in a period of heavy milk production a much higher percentage of the food elements offered in the ration would be assimilated than in a period when no milk was being secreted. Likewise a smaller relative amount, though perhaps a greater absolute quantity, would be utilized from a ration high in calcium and phosphorus than from one poor in these elements.

EXPERIMENTAL

The animals used in this investigation were grade Holsteins. Attention is called to the fact that all were heavy milkers and in some cases unusual ones. Most of them were giving in the neighborhood of 25 kgm. per day at the height of lactation and one, M 253, gave over 35 kgm. per day. Cows M 218, M 234, M 242, and M 281 received a ration consisting of timothy hay, corn silage, and a grain mixture containing 4 parts yellow corn, 2 parts old process linseed oil meal, 1 part oats, and 1 per cent salt after first calving. Beginning at first calving, cows M 222, M 227, M 236, M 240, and M 253 were fed the above ration supplemented with bone flour as 3 per cent of the grain mixture while M 329, M 333, M 334 and M 335 received the same proportion of $CaCO_3$ instead of bone flour. Cows M 214, M 216, M 217, and M 257 were fed alfalfa hay in place of timothy. After first freshening a grain mixture consisting of 4 parts yellow corn, 1 part old process linseed oil meal, 1 part oats, and 1 per cent salt was fed. The three rations were planned to differ only in mineral content, the amounts of protein supplied being about alike. These rations were fed through several years so that the question of the animals adapting themselves to a new diet is not a factor in these results.

The hays used were of unknown history, selected on the basis of color and purity. Most of the timothy used in this work was a high grade No. 2 (United States Standard).

The alfalfa hay used for the main dairy herd was fed to the

TABLE 1

Average daily calcium and phosphorus metabolism of cows fed a basic ration of timothy hay, silage, and grain

ANIMAL NUMBER	PERIOD	CALCIUM								PHOSPHORUS							
		Average daily milk	Outgo				Intake	Balance	Used	Ca/P in food	Outgo				Intake	Balance	Used
			Feces	Urine	Milk	Total					Feces	Urine	Milk	Total			
		kgm.	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*		*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*
218	Apr. 7–14/27	28.8*	18.76	1.97	27.62	48.35	43.29	−5.06	52.1	0.80	31.92	0.33	21.58	53.83	54.08	0.25	40.4
	Oct. 8–15/28	23.1*	24.31	1.09	23.13	48.53	50.55	2.02	49.8	0.88	23.72	0.08	17.81	41.61	57.12	15.51	58.3
	Aug. 17–24/26	15.8	20.93	2.10	16.76	39.79	40.72	0.93	43.4	0.83	30.64	0.14	14.58	45.36	49.01	3.65	37.2
	Feb. 14–21/27	Dry	13.35	0.91		14.26	26.92	12.66	47.0	1.03	18.17	0.21		18.38	26.24	7.86	30.0
M 234	May 17–24/27	26.7*	17.21	1.76	30.19	49.16	37.38	−11.78	49.3	0.78	28.88	1.30	22.17	52.35	47.71	−4.64	36.7
	Jun. 11–18/28	26.5*	21.46	2.52	30.51	54.49	56.61	2.12	57.6	0.98	30.82	0.16	23.35	54.33	57.96	3.63	46.5
	Dec. 15–22/26	8.0	15.71	1.48	8.07	25.26	30.95	5.69	44.5	1.10	18.26	0.20	6.72	25.17	28.08	2.91	34.3
	Mar. 11–18/28	Dry	16.67	1.04		17.71	27.70	9.99	36.1	1.14	20.43	0.23		20.66	24.20	3.54	14.6
M 242	Aug. 17–24/26	24.8*	28.24	1.48	28.05	57.77	48.92	−8.85	39.2	0.78	43.33	1.65	23.80	68.78	62.99	−5.79	28.6
	Jan. 4–11/27	13.5*	21.67	0.97	14.59	37.23	37.83	0.60	40.2	0.99	26.30	1.53	13.10	40.93	38.32	−2.61	27.4
	Jul. 25–Aug. 1/27	Dry*	27.67	0.74		28.41	34.41	6.00	17.4	1.29	18.71	5.69		24.40	26.67	2.27	8.5
M 281	Jul. 5–12/27	27.6*	25.35	1.29	30.09	56.73	41.34	−15.39	35.6	0.84	30.20	0.22	23.19	53.61	49.30	−4.31	38.3
	Oct. 8–15/28	27.4*	21.40	0.98	27.40	49.78	41.91	−8.87	46.6	0.92	21.97	0.10	21.65	43.72	45.70	1.98	51.7
	Jan. 4–11/27	8.7	13.33	1.07	8.92	23.32	29.90	6.58	51.8	1.04	17.85	0.41	8.30	26.56	28.81	2.25	36.6
	May 17–24/27	Dry	20.17	0.84		21.01	31.94	10.93	34.2	1.38	21.18	0.12		21.30	24.57	3.27	13.3
M 258†	Mar. 24–31/28	24.4	29.01	2.67	31.00	62.68	60.18	−2.50	47.4	1.21	28.68	0.10	20.99	49.77	49.66	−0.11	42.0
	Oct. 22–29/28	8.5	21.04	0.77	9.88	31.69	31.44	−0.25	30.6	0.98	20.19	0.10	6.98	27.27	32.02	4.75	36.6

M 259†	Mar. 24–31/28	27.9*	24.93	1.13	33.51	59.57	66.17	6.60	60.6	1.28	22.94	0.12	26.53	49.59	51.51	1.92	55.2
	Oct. 22–29/28	6.8	22.65	0.50	8.01	31.16	29.36	−1.80	21.2	1.04	15.18	0.10	6.04	21.32	28.19	6.87	45.8
M 266†	Apr. 1–8/29	28.2*	28.55	2.16	28.18	58.89	62.02	3.13	50.4	0.81	49.48	0.20	27.06	76.74	76.45	−0.29	35.0
	Mar. 2–9/28	27.5*	27.84	1.29	36.03	65.16	57.71	−7.45	49.5	1.05	29.88	0.08	26.68	56.64	55.24	−1.40	45.9
	Oct. 22–29/28	1.6	20.58	1.43	2.29	24.30	31.68	7.38	30.5	1.07	16.03	0.12	1.54	17.69	29.72	12.03	45.7

* Not pregnant.

† These animals had pasture in season but were on the basal ration during metabolism.

TABLE 2

Average daily calcium and phosphorus metabolism of cows fed a basic ration of timothy hay, silage, and grain with bone flour as a mineral supplement

ANIMAL NUMBER	PERIOD	CALCIUM								PHOSPHORUS							
		Average daily milk	Outgo				Intake	Balance	Used	Ca/P in food	Outgo				Intake	Balance	Used
			Feces	Urine	Milk	Total					Feces	Urine	Milk	Total			
		kgm.	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*		*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*
M 222	Jan. 16–23/28	28.6*	91.99	2.63	36.01	130.63	129.81	−0.82	27.1	1.23	84.85	0.21	27.73	112.79	105.76	−7.03	19.6
	May 18–25/28	21.7	76.01	4.63	24.97	105.61	120.59	14.98	33.1	1.69	41.73	0.22	18.89	60.84	71.58	10.74	41.4
	Apr. 21–28/27	17.6	54.80	4.84	19.49	79.13	88.86	9.73	32.9	1.24	57.05	0.08	16.86	73.99	71.92	−2.57	19.9
	Dec. 26–Jan. 2/29	Dry	52.86	2.27		55.13	64.61	9.48	14.7	1.47	41.87	0.15		42.02	44.12	2.10	4.8
M 237	Feb. 14–21/27	28.7*	86.49	6.65	29.67	122.81	124.14	1.33	25.0	1.20	83.72	0.51	26.33	110.56	103.80	−6.76	18.9
	Aug. 17–24/26	8.9	54.31	2.09	8.90	65.30	70.71	5.41	20.2	1.09	47.14	1.34	8.24	56.72	64.85	8.13	25.2
	Dec. 15–22/26	Dry	37.59	2.15		39.73	53.50	13.77	25.7	1.25	31.01	0.21		31.22	42.84	11.62	27.1
M 236	June 11–18/28	30.3*	96.82	3.12	31.53	131.47	150.40	18.93	33.6	1.47	63.39	0.65	20.92	84.96	102.05	17.09	37.2
	July 5–12/27	23.7*	78.68	3.57	26.77	109.02	104.79	−4.23	21.5	1.15	67.75	3.47	19.43	90.65	91.44	0.79	22.1
	Dec. 17–24/27	9.5	62.03	1.96	11.45	75.44	81.16	5.72	21.2	1.59	42.66	0.01	6.87	49.54	50.89	1.35	16.2
	Apr. 7–14/27	Dry	36.60	1.43		38.03	54.30	16.27	29.9	1.27	34.50	0.10		34.60	42.65	8.05	18.9
M 240	July 5–12/27	23.3*	85.75	2.71	26.60	115.06	106.73	−8.32	17.1	1.12	75.36	0.73	21.46	97.55	95.12	−2.43	20.0
	Oct. 3–10/27	17.1	71.37	3.17	19.71	94.25	99.53	5.28	25.1	1.42	60.20	0.37	15.77	76.34	69.89	−6.45	13.3
	Jan. 4–11/27	8.1	45.16	3.48	9.45	58.09	67.09	9.00	27.5	1.30	42.71	0.50	7.79	51.00	51.67	0.67	16.4
	May 17–24/27	Dry	40.60	1.09		41.69	52.76	11.08	21.0	1.49	34.52	0.22		34.74	35.47	0.73	2.1
M 253	Mar. 11–18/29	36.6*	96.42	1.40	48.29	146.11	148.29	2.18	34.0	1.27	76.57	0.19	36.95	113.71	116.82	3.11	34.3
	Apr. 1–8/29	34.4*	93.81	1.89	42.70	138.40	139.75	1.35	31.5	1.23	77.90	0.21	34.78	112.89	113.36	0.47	31.1
	Mar. 2–9/28	28.1*	103.11	2.63	35.84	141.58	131.22	−10.36	19.4	1.26	85.97	0.09	25.68	111.14	104.01	−7.13	17.8
	Mar. 11–18/27	12.0*	56.66	4.30	12.83	73.79	81.50	7.71	25.2	1.21	56.15	0.21	10.99	67.35	67.09	−0.26	16.0
	Oct. 3–10/27	Dry	54.54	2.42		56.96	63.95	6.99	10.9	1.61	44.97	0.43		45.40	39.63	−5.77	

* Not pregnant.

TABLE 3

Calcium and phosphorus metabolism of cows fed a basic ration of timothy hay, silage and grain with calcium carbonate as a mineral supplement

ANIMAL NUMBER	PERIOD	CALCIUM								PHOSPHORUS							
		Average daily milk	Outgo				Intake	Balance	Used	Ca/P in food	Outgo				Intake	Balance	Used
			Feces	Urine	Milk	Total					Feces	Urine	Milk	Total			
		kgm.	grams	grams	grams	grams	grams	grams	per cent		grams	grams	grams	grams	grams	grams	per cent
M 335	Apr. 1–8/29	28.4*	124.32	0.72	28.13	153.17	173.97	20.80	28.1	2.52	34.14	0.20	27.28	61.62	69.04	7.42	50.2
	Mar. 2–9/28	22.7*	110.84	0.86	24.28	135.98	154.27	18.29	27.5	3.65	29.17	0.08	20.65	49.90	42.21	−7.69	30.7
	Jul. 9–16/28	16.0	103.76	2.00	18.05	123.81	132.47	8.66	20.1	3.18	22.59	0.09	15.97	38.65	41.58	2.93	45.4
	Dec. 26–Jan. 2/29	Dry	51.13	1.14		52.27	65.39	13.12	20.0	2.49	19.86	0.06		19.92	26.21	6.29	24.0
M 329	Jan. 16–23/28	19.4*	100.41	2.18	24.00	126.59	152.15	25.56	32.5	3.52	21.42	0.09	21.49	43.00	43.16	0.16	50.1
	Jul. 9–16/28	11.5	85.36	4.01	13.75	103.12	113.33	10.21	21.1	3.19	21.02	0.17	11.92	33.11	35.42	2.31	40.1
	Dec. 26–Jan. 2/29	Dry	49.57	1.25		50.82	65.37	14.55	22.2	2.49	21.77	0.13		21.90	26.21	4.31	16.4
M 333	Mar. 24–31/28	18.3*	104.31	1.58	21.83	127.72	154.94	27.22	31.6	3.76	20.45	0.11	17.24	37.80	41.14	3.34	50.0
	Jul. 9–16/28	13.1	84.67	1.98	14.84	101.49	118.68	18.19	27.6	3.18	20.71	0.15	12.35	33.21	37.54	4.33	44.4
M 334	Jun 11–18/28	19.3*	101.83	2.18	23.70	127.71	130.27	2.56	20.1	3.07	25.10	0.23	17.15	42.48	42.37	−0.11	40.2
	Aug. 20–27/29	10.8*	81.15	1.38	13.43	95.96	116.43	20.47	29.1	2.49	30.22	0.40	10.50	41.12	46.74	5.62	34.4

* Not pregnant.

TABLE 4

Average daily calcium and phosphorus metabolism of cows fed alfalfa hay, silage and grain

ANIMAL NUMBER	PERIOD	CALCIUM								PHOSPHORUS							
		Average daily milk	Outgo				Intake	Balance	Used	Ca/P in food	Outgo				Intake	Balance	Used
			Feces	Urine	Milk	Total					Feces	Urine	Milk	Total			
		kgm.	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*		*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*per cent*
M 214	Sept. 13–20/26	20.9*	42.39	0.82	25.87	69.08	73.89	4.81	41.5	1.84	21.03	0.55	18.95	40.53	40.12	−0.41	46.2
	Feb. 14–21/27	13.3	84.89	2.13	13.33	100.35	100.36	0.01	13.3	3.65	15.77	0.19	9.91	25.87	27.44	1.57	41.8
	Jul. 15–22/27	Dry	85.43	1.01		86.44	97.61	11.17	11.4	4.46	17.75	0.08		17.83	21.88	4.13	18.9
M 216	Sept. 13–20/26	27.2*	69.14	1.16	33.58	103.88	106.71	2.83	34.1	2.26	25.69	0.16	24.84	50.69	47.23	−3.46	45.2
M 217	Jan. 16–23/28	28.2*	47.49	1.11	36.47	85.07	85.17	0.10	42.9	1.86	18.67	0.83	27.14	46.63	45.71	−0.92	57.3
	May 18–25/28	17.9	106.26	0.10	20.92	127.28	131.01	3.73	18.8	3.27	22.45	0.31	15.38	38.14	40.06	1.92	43.2
	Apr. 21–28/27	10.0	81.00	0.39	10.64	92.03	94.72	2.69	14.1	3.75	19.58	0.24	10.24	30.06	25.23	−4.83	21.4
	Sept. 13–20/26	Dry	74.67	0.49		75.16	87.99	12.83	14.6	4.33	16.46	0.19		16.65	20.31	3.66	18.0
M 257	Dec. 15–22/26	20.3*	69.87	1.24	23.00	94.11	90.33	−3.78	21.3	1.87	26.32	0.20	20.56	47.08	48.21	1.13	44.9
	Apr. 21–28/27	6.5	76.23	0.61	6.61	83.45	92.45	9.00	16.9	4.49	16.24	0.12	5.57	21.93	20.59	−1.34	20.5
	Oct. 3–10/27	Dry	114.90	1.07		115.97	119.55	3.58	3.0	9.29	11.97	0.27		12.24	12.87	0.63	4.8

* Not pregnant.

animals in this investigation. No effort was made to procure a certain grade of alfalfa. The alfalfa used in this work was usually lower in grade than the timothy.

Method of procedure

Calcium and phosphorus balances were obtained at various times throughout the lactation periods and when the cows were dry. Seven day collections were made during heavy, medium or light production, and again during the rest period. Quantities of feed were graduated to keep the appetite keen so that all the food would be cleaned up. Shavings were used as bedding during the preliminary period and canvas mattresses during the collection period. The metabolism stalls were fitted with special mangers to prevent loss of feed. The cows were watered from a tub three times daily, using college water which contained 0.008 per cent calcium. Attendants collected the urine and feces separately by means of pails and shovels. The cows were exercised for thirty minutes each day regardless of weather conditions.

The feces samples were analyzed daily. The urines and milks were composited and aliquots, preserved with thymol and formalin respectively, were saved for analysis.

The methods of analysis used in this experiment were the same as in previous work (10).

The average daily milk yields, and average daily calcium and phosphorus metabolism figures are shown in tables 1, 2, 3 and 4.

The percentage of "calcium used" is the algebraic sum of the "calcium balance" and the calcium in the milk. From the standpoint of the work in hand, milk is considered as a constituent of the body. The "phosphorus used" was computed in the same way. These correspond to Meigs' values for "assimilation" (4).

DISCUSSION

Calcium and phosphorus balance

We are quite unable to account for the results of Forbes and his associates (1) which indicated that balances were negative with cows producing over 5 kgm. of milk per day as our animals

frequently maintained a positive balance while producing from 5 to 7 times that amount. Our results confirm those of Hart and his associates (5) (6) of Perkins and Monroe (9) and of the Vermont Experiment Station (8), all of whom report positive balances with cows giving only slightly less milk than ours. Our results assembled in table 1 indicate that cows receiving a ration of timothy hay, corn silage and grain without a mineral supplement received sufficient calcium and phosphorus for the production of at least 10,000 pounds of milk per year. These cows were on negative balance during heavy production, but later during medium production and when dry stored enough calcium to compensate for the loss during heavy production. It should be emphasized however that in this experiment negative balances or equilibrium was the rule during the first or high production period.

When the basal ration was supplemented by 3 per cent of bone flour or calcium carbonate, positive balances were secured with most cows even during the heavy production period. However, two animals showed negative calcium balances and three negative phosphorus balances in later periods. We are inclined to accept the negative values of the high milking periods as evidence of the failure on the part of the animal to meet the demand of milk production, but subsequent negative balances were probably caused by decreased absorption because of digestive disturbances that prevailed throughout the herd at various times.

The replacement of timothy hay by alfalfa accomplished even more than the addition of the mineral supplement in so far as the calcium balance was concerned. In only one case was there a negative value for calcium balance.

A survey of all these tables reveals the fact that on any of the rations, heavily milking cows were in positive balance most of the time, while on a ration designed to reduce losses, the situation was decidedly favorable for continuous positive utilization of both elements.

Calcium and phosphorus utilization

Meigs and his associates (4) summarized the calcium assimilation figures reported in his experiments and those obtained at

the Wisconsin Experiment Station and concluded "that the average proportion of calcium assimilated by milking cows from fairly well cured hay is somewhere in the neighborhood of 20 per cent of the intake. About the same percentage of calcium is assimilated from timothy hay with its low calcium content as from alfalfa with its much higher one." Our results fail to confirm either of these conclusions. The cows in our experiments receiving timothy hay, corn silage and grain, a ration low in calcium, utilized (with the exception of one low value of 17.4 per cent from 34.2 to 57.6 per cent of the ingested calcium. The one low value was recorded for cow M 242 who failed to conceive and whose need for calcium was therefore reduced. Had she required more for the production of a fetus she would probably have shown a value as high as the others. All cows showed a tendency to increase the assimilation during high productivity when the demands were the greatest. Including all values, the average for the seven cows on this ration were respectively 48.1, 46.9, 32.3, 42.1, 39.0, 40.9 and 40.1 per cent of the food calcium.

When alfalfa was used instead of timothy the calcium intake was more than doubled and the assimilation averages were 22.1, 22.6, 34.1 and 13.7 per cent respectively for the four cows. Individual percentages during the heaviest milking period were much above this as they were in the timothy group. Thus, contrary to Meigs contention, the average proportion assimilated was greatly reduced when calcium-rich alfalfa was used as compared with the proportion assimilated when the calcium-poor timothy was fed.

The groups given the basal ration supplemented with bone flour and calcium carbonate lend further support to our conclusion that the intake and need of the animal determines the proportion of calcium that will be assimilated from the food. In this experiment wherein the intake was greatly increased over that of the basal ration, the averages of calcium assimilated dropped to 27.0, 23.6, 26.6, 22.7, 24.2, 24.0, 25.3, 29.6 and 24.6 per cent.

What has been said of calcium is equally true of phosphorus. The relation between "intake" and "utilization" is particularly well shown in tables 2, 3 and 4. In the experiment concerned

TABLE 5

Ages, stage of lactation and gestation period

ANIMAL NUMBER	DATE OF BIRTH	METABOLISM PERIOD	DATE OF LAST CALVING	DAYS PREGNANT
M 218	Sept. 15/22	Apr. 7–14/27	Feb. 24/27	None
		Oct. 8–15/28	June 17/28	None
		Aug. 17–24/26	Dec. 15/25	76
		Feb. 14–21/27	Dec. 15/25	257
M 234	Apr. 6/23	May 17–24/27	Apr. 21/27	None
		June 11–18/28	Apr. 19/28	None
		Dec. 15–22/26	Apr. 16/26	150
		Mar. 11–18/28	Apr. 21/27	236
M 242	May 17/23	Aug. 17–24/26	July 23/26	None
		Jan. 4–11/27	July 23/26	None
		July 25–Aug. 1/27	July 23/26	None
M 281	June 18/24	July 5–12/27	June 13/27	None
		Oct. 8–15/28	Sept. 8/28	None
		Jan. 4–11/27	June 6/26	133
		May 17–24/27	June 6/26	246
M 258	Dec. 8/23	Mar. 24–31/28	Feb. 11/28	None
		Oct. 22–29/28	Feb. 11/28	172
M 259	Dec. 18/23	Mar. 24–31/28	Feb. 11/28	None
		Oct. 22–29/28	Feb. 11/28	116
M 266	Jan. 12/24	Apr. 1–8/29	Jan. 26/29	None
		Mar. 2–9/28	Jan. 20/28	None
		Oct. 22–29/28	Jan. 20/28	189
M 222	Oct. 14/22	Jan. 16–23/28	Dec. 18/27	None
		May 18–25/28	Dec. 18/27	21
		Apr. 21–28/27	Oct. 18/26	35
		Dec. 26–Jan. 2/29	Dec. 18/27	253
M 227	Dec. 23/22	Feb. 14–21/27	Jan. 9/27	None
		Aug. 17–24/26	Jan. 3/26	133
		Dec. 15–22/26	Jan. 3/26	253
M 236	Apr. 14/23	June 11–18/28	May 9/28	None
		July 5–12/27	May 11/27	None
		Dec. 17–24/27	May 11/27	118
		Apr. 7–14/27	Apr. 3/26	251

TABLE 5—*Concluded*

ANIMAL NUMBER	DATE OF BIRTH	METABOLISM PERIOD	DATE OF LAST CALVING	DAYS PREGNANT
M 240	May 7/23	July 5–12/27	June 7/27	None
		Oct. 3–10/27	June 7/27	None
		Jan. 4–11/27	May 21/26	118
		May 17–24/27	May 21/26	251
M 253	Oct. 19/23	Mar. 11–18/29	Feb. 17/29	None
		Apr. 1–8/29	Feb. 17/29	None
		Mar. 2–9/28	Jan. 26/28	None
		Mar. 11–18/27	Oct. 5/26	None
		Oct. 3–10/27	Oct. 5/26	208
M 335	Mar. 7/26	Apr. 1–8/29	Jan. 20/29	None
		Mar. 2–9/28	Jan. 31/28	None
		July 9–16/28	Jan. 31/28	80
		Dec. 26–Jan. 2/29	Jan. 20/29	250
M 329	Dec. 9/25	Jan. 16–23/28	Dec. 5/27	None
		July 9–16/28	Dec. 5/27	61
		Dec. 26–Jan. 2/29	Dec. 5/27	231
M 333	Feb. 25/26	Mar. 24–31/28	Feb. 4/28	None
		July 9–16/28	Feb. 4/28	62
M 334	Feb. 28/26	June 11–18/28	May 17/28	None
		Aug. 20–27/29	May 21/29	None
M 214	July 28/22	Sept. 13–20/26	Aug. 6/26	None
		Feb. 14–21/27	Aug. 6/26	107
		July 15–22/27	Aug. 6/26	258
M 216	Aug. 16/22	Sept. 13–20/26	Aug. 10/26	None
M 217	Aug. 20/22	Jan. 16–23/28	Nov. 29/27	None
		May 18–25/28	Nov. 29/27	80
		Apr. 21–28/27	Oct. 22/26	61
		Sept. 13–20/26	Sept. 20/25	237
M 257	Nov. 10/23	Dec. 15–22/26	Nov. 20/26	None
		Apr. 21–28/27	Nov. 20/26	78
		Oct. 3–10/27	Nov. 20/26	243

TABLE 6

Composition of feeds

ANIMAL NUMBER	METABOLISM PERIOD	HAY		GRAIN		SILAGE	
		Ca	P	Ca	P	Ca	P
M 218	Apr. 7–14/27	0.510	0.230	0.177	0.400	0.083	0.054
	Oct. 8–15/28	0.317	0.105	0.210	0.479	0.087	0.059
	Aug. 17–24/26	0.303	0.087	0.176	0.434	0.086	0.092
	Feb. 14–21/27	0.277	0.191	0.151	0.375	0.071	0.041
M 234	May 17–24/27	0.320	0.107	0.149	0.399	0.082	0.042
	June 11–18/28	0.336	0.088	0.181	0.438	0.131	0.049
	Dec. 15–22/26	0.290	0.110	0.169	0.394	0.084	0.045
	Mar. 11–18/28	0.320	0.135	0.149	0.400	0.083	0.048
M 242	Aug. 17–24/26	0.303	0.083	0.176	0.434	0.086	0.092
	Jan. 4–11/27	0.272	0.148	0.174	0.399	0.095	0.051
	July 25–Aug. 1/27	0.320	0.116	0.181	0.367	0.128	0.095
M 281	July 5–12/27	0.258	0.126	0.197	0.419	0.069	0.042
	Oct. 8–15/28	0.317	0.105	0.172	0.384	0.087	0.059
	Jan. 4–11/27	0.272	0.148	0.174	0.399	0.095	0.051
	May 17–24/27	0.320	0.107	0.149	0.399	0.082	0.042
M 258	Mar. 24–31/28	0.455	0.091	0.248	0.389	0.100	0.037
	Oct. 22–29/28	0.226	0.149	0.164	0.422	0.097	0.056
M 259	Mar. 24–31/28	0.455	0.091	0.248	0.389	0.100	0.037
	Oct. 22–29/28	0.226	0.149	0.164	0.422	0.097	0.056
M 266	Apr. 1–8/29	0.340	0.139	0.240	0.590	0.123	0.066
	Mar. 2–9/28	0.346	0.142	0.201	0.402	0.111	0.037
	Oct. 22–29/28	0.226	0.149	0.164	0.422	0.097	0.056
M 222	Jan. 16–23/28	0.418	0.137	0.854	0.890	0.191	0.043
	May 18–25/28	0.360	0.082	1.005	0.765	0.118	0.037
	Apr. 21–28/27	0.280	0.126	0.839	0.830	0.105	0.048
	Dec. 26–Jan. 1/29	0.266	0.080	1.010	0.891	0.091	0.057
M 227	Feb. 14–21/27	0.277	0.191	0.830	0.760	0.071	0.041
	Aug. 17–24/26	0.303	0.087	0.844	0.928	0.086	0.092
	Dec. 15–22/26	0.290	0.110	0.825	0.897	0.080	0.045
M 236	June 11–18/28	0.336	0.088	1.030	0.843	0.131	0.049
	July 5–12/27	0.258	0.126	0.863	0.860	0.069	0.042
	Dec. 17–24/27	0.299	0.148	0.840	0.845		
	Apr. 7–14/27	0.510	0.230	0.847	0.865	0.083	0.054

TABLE 6—*Concluded*

ANIMAL NUMBER	METABOLISM PERIOD	HAY		GRAIN		SILAGE	
		Ca	P	Ca	P	Ca	P
M 240	July 5–12/27	0.258	0.126	0.863	0.860	0.069	0.042
	Oct. 3–10/27	0.357	0.123	0.838	0.811	0.130	0.041
	Jan. 4–11/27	0.272	0.148	0.854	0.799	0.095	0.051
	May 17–24/27	0.320	0.107	0.149	0.399	0.082	0.042
M 253	Mar. 11–18/29	0.281	0.156	1.050	0.924	0.117	0.067
	Apr. 1–8/29	0.340	0.139	0.950	0.911	0.123	0.066
	Mar. 2–9/28	0.346	0.142	0.878	0.850	0.111	0.037
	Mar. 11–18/27	0.320	0.135	1.115	0.467	0.164	0.096
	Oct. 3–10/27	0.357	0.123	0.838	0.811	0.130	0.041
M 335	Apr. 1–8/29	0.340	0.139	1.260	0.499	0.123	0.066
	Mar. 2–9/28	0.346	0.142	1.440	0.850	0.111	0.037
	July 9–16/28	0.314	0.089	1.320	0.446	0.151	0.044
	Dec. 26–Jan. 2/29	0.266	0.080	1.130	0.428	0.091	0.057
M 329	Jan. 16–23/28	0.418	0.137	1.310	0.396	0.191	0.043
	July 9–16/28	0.314	0.089	1.320	0.446	0.151	0.044
	Dec. 26–Jan. 2/29	0.266	0.080	1.130	0.428	0.091	0.057
M 333	Mar. 24–31/28	0.455	0.091	1.320	0.362	0.100	0.037
	July 9–16/28	0.314	0.089	1.320	0.446	0.151	0.044
M 334	June 11–18/28	0.336	0.088	1.290	0.435	0.131	0.049
	Aug. 20–27/29	0.386	0.161	1.297	0.490	0.107	0.062
M 214	Sept. 13–20/26	1.651	0.143	0.114	0.362	0.096	0.080
	Feb. 14–21/27	1.412	0.177	0.115	0.315	0.071	0.041
	July 15–22/27	1.175	0.161	0.144	0.335	0.174	0.056
M 216	Sept. 13–20/26	1.651	0.143	0.114	0.362	0.096	0.080
M 217	Jan. 16–23/28	1.580	0.163	0.131	0.340	0.191	0.043
	May 18–25/28	1.793	0.143	0.200	0.429	0.118	0.039
	Apr. 21–28/27	1.270	0.186	0.120	0.341	0.105	0.048
	Sept. 13–20/26	1.651	0.143	0.114	0.362	0.096	0.080
M 257	Dec. 15–22/26	1.428	0.167	0.127	0.337	0.080	0.045
	Apr. 21–28/27	1.270	0.186	0.120	0.341	0.105	0.048
	Oct. 3–10/27	1.740	0.140	No grain		0.130	0.040

in table 2, the basal ration used in experiment I was supplemented with bone flour, causing an increased intake of both calcium and phosphorus. There was a corresponding fall in assimilation values. In experiment IV the substitution of alfalfa for timothy an in experiment III the addition of calcium carbonate increased the calcium intake but left the phosphorus values about as they were in experiment I. There was a marked reduction in the calcium assimilated but the proportion of phosphorus utilized remained practically the same.

The relative importance of total intake of Ca or P and the Ca/P ratio on the amounts of Ca and P used

Many studies have been made on this subject in relation to the production of rickets and malnutrition in growing animals and some work has been done on the importance of the Ca/P ratio in the utilization of these elements by high producing cows (11) (12). 'In order to get some idea of the relative importance of the total intake of calcium and phosphorus and their ratio in the food data were collected from several sources besides the present paper and treated statistically. A total of 132 experiments were secured from ten different publications (1) (2) (4) (5) (11) (12), embracing animals on various rations in positive or negative balance, pregnant and non-pregnant, and living under different conditions. An arbitrary minimum of 15 kgm. of milk per day was taken to define a heavily milking cow and all the subjects considered were giving this amount of milk or more. The following table shows the indices of correlation.

	INTAKE	Ca/P
Per cent Ca used	0.9053 ±0.0107	0.5704 ±0.0396
Per cent P used	0.8089 ±0.0203	0.3913 ±0.0497

It is evident that the actual intake has a much greater significance than the ratio as regards the utilization of calcium and phosphorus.

SUMMARY

1. A series of calcium and phosphorus balances on cows when in heavy, in medium, or in low milk production and when dry,

on a ration of timothy hay, corn silage, and grain indicates that the ration supplied sufficient calcium and phosphorus for the production of at least 10,000 pounds of milk a year. During the height of production the animals were frequently in negative balance but subsequently positive balances made up the loses.

2. Positive calcium and phosphorus balances were obtained in heavily milking cows when the above ration of timothy hay, corn silage, and grain was supplemented with bone flour. A cow producing 80 pounds of milk a day showed positive calcium and phosphorus balances.

3. Positive calcium balances were obtained in heavily milking cows on a ration of alfalfa, silage and grain.

4. Cows fed a low calcium ration utilized calcium more efficiently than when the ration was high in this element.

5. There was a tendency for cows in heavy milk production to utilize both calcium and phosphorus more efficiently than when in low production or during the dry period.

6. The total intake of calcium or phosphorus has a greater significance in the utilization of these elements than has the Ca/P ratio in the food.

We wish to thank Professor S. E. Crowe for assistance in the statistical treatment of the results.

REFERENCES

(1) Forbes, E. B., et al. 1916–1922 The mineral metabolism of the milch cow, I–IV. Ohio Agr. Exp. Sta. Bulletins Nos. 295, 308, 330 and 363.

(2) Hart, E. B., Steenbock, H., Hoppert, C. A., Bethke, R. M., and Humphrey, G. C. 1922 Dietary factors influencing calcium assimilation, III. Jour. Biol. Chem., liv, 75.

(3) Meigs, E. B., Blatherwick, N. R., and Cary, C. A. 1919 Further contributions to the physiology of phosphorus and calcium metabolism of dairy cows. Jour. Biol. Chem., xl, 469.

(4) Meigs, E. B., Turner, W. H., Harding, T. S., Hartman, A. M., and Grant, F. M. 1926 Calcium and phosphorus metabolism in dairy cows. Jour. Agr. Res., xxxii, 833.

(5) Hart, E. B., Steenbock, H., Hoppert, C. A., and Humphrey, G. C. 1922 Dietary factors influencing calcium assimilation. II. Jour. Biol. Chem., liii, 21.

(6) Hart, E. B., Steenbock, H., Scott, H., and Humphrey, G. C. 1927 Dietary factors influencing calcium assimilation, VIII. Jour. Biol. Chem., lxxi, 263.

(7) Miller, H. G., Brandt, P. M., and Jones, R. C. 1924 Mineral metabolism studies with dairy cattle. Amer. Jour. Physiol., lxix, 169.

(8) Maintenance requirements for dairy cows. 1925–26 Vermont Agr. Exp. Sta., 39th Annual Report, 11.

(9) Perkins, A. E., and Monroe, C. F. 1924 Effect of high and low protein content on the digestibility and metabolism of dairy rations. Ohio Agr. Exp. Sta. Bul. 376.

(10) Robinson, C. S., Huffman, C. F., and Mason, M. F. 1929 The results of the ingestion of certain calcium salts and of lactose. Jour. Biol. Chem., lxxxiv, 257.

(11) Turner, W. A., Harding, T. S., and Hartman, A. M. 1927 The relative assimilation by dairy cows of clover and alfalfa hays and of rations of different calcium and phosphorus content. Jour. Agr. Res., xxxv, 625.

(12) Turner, W. A., and Hartman, A. M. 1929 Calcium and phosphorus metabolism in dairy cows, III. Jour. Nutrition, 1, 445.

A STUDY OF THE BACTERIAL CONTENT OF THE FORE MILK OF COWS*

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It has been shown by certain investigators that the fore milk has a higher bacterial count than the middle milk. Little information seems to be available on the number of streams which should constitute the fore milk. This work has, therefore, been outlined with the hope of throwing a little more light on this subject.

Trout (10) using the first three to five streams as the fore milk found an average of 3,900 bacteria per cubic centimeter. The remainder of the milk showed a count of 890 per cubic centimeter. Harding and Wilson (5) reported the average bacterial count based on 1,230 samples from 78 different cows to be 428 bacteria per cubic centimeter. The samples were obtained at the close of the milking. Copeland and Olsen (4) determined the number of bacteria in milk from each quarter of the udder of 40 cows, the samples being taken after approximately one-third of the milk had been drawn. The average number was 1,540 per cubic centimeter. Breed (3) found an average bacterial content of 964 per cubic centimeter for 45 samples of strippings milk drawn directly into sterile test tubes from individual quarters.

In a series of comparisons von Freudenreich (11) found the fore milk averaged 6,000 per cubic centimeter, the middle milk 1,341, and the strippings 769. Stocking (9) using streams 1 and 2; 5 and 6; 9 and 10; 13 and 14 and the strippings showed that in general there is a decrease in the bacterial count as the milking proceeds. Orla-Jensen (7) secured milk in sterile tubes after washing the udder and teats and found the fore milk from 4 quarters contained 16,000 bacteria per cubic centimeter; the middle milk 480, and the strippings 360. Copeland and Olsen (4)

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determined the number of bacteria in the fore milk, middle milk, and strippings from each quarter of 8 cows. The fore milk averaged 5,989 per cubic centimeter, the middle milk 577 and the strippings 415.

Russell (8) found that the milk drawn first from each duct contained 2,800 bacteria per cubic centimeter, while the remainder averaged only 330 per cubic centimeter. Work done by Backhaus and Appel (2) showed the germ count to decrease regularly as the milking progressed, and in a few cases, the last portion was sterile. The authors recommended the separation of the first quarter from the last three quarters of the milking. Backhaus and Cronheim (1) used the milk of 8 cows, which was drawn with care into a sterile pail. Rejecting the first 5 spurts of milk, an average bacterial count of 6,600 per cubic centimeter was obtained. Lux (6) did work concerning the total bacterial counts of milk at hour intervals of from 1 to 6 hours, and gave the species of bacteria commonly found.

The present experiment was started October, 1928 and continued through May, 1929. The samples of milk were obtained from the dairy herd at the University of Maryland. The udders of the cows were first washed and dried after which the milk was drawn into sterile Baltimore milk sample bottles. Each sample was a mixture of 4 streams, one being taken from each quarter of the udder. Five such samples were obtained from the first 5 streams and were arbitrarily considered as the fore milk. The next 5 streams from each quarter were discarded and the eleventh stream was used for the sample of middle milk. The samples were placed in the ice box at a temperature of between 40° and 44°F. for 18 hours before plating. The time between drawing the samples and placing them in the ice box was about 15 minutes. The data for the bacterial counts were based on the examination of 100 samples for each stream.

Standard beef extract agar was used as a medium. Recognizing that the medium is not particularly adapted for growing udder bacteria, it would, therefore, not be expected to find as high counts as on the special media. On the other hand, it is believed that in

using standard beef extract agar, a medium is being used which should produce counts comparable with those obtained in standard routine milk analysis.

Dilutions of 1:10, 1:100 and 1:1000 were used in plating the milk. Plating, incubation, and counting were done according to the Standard Methods of Milk Analysis (table 1).

The reduction in bacterial content of stream 11 over stream 5 is so slight that the discarding of the sixth to the tenth streams would seem to be a questionable procedure, from a practical

TABLE 1

Average bacterial counts per cubic centimeter

(Plated on standard beef extract agar)

STREAM NUMBER	AVERAGE BACTERIAL COUNT
1	1,275
2	927
3	705
4	647
5	551
11	449

Average fore milk count was 821 bacteria per cubic centimeter.
Average middle milk count was 449 bacteria per cubic centimeter.

standpoint. The data show that the first 5 streams only may be discarded as the fore milk for the reason that they carry increased numbers of bacteria.

REFERENCES

(1) BACKHAUS AND CRONHEIM, W. 1897. Containing milk under aseptic conditions. Ber. Landw. Inst. Univ. Konigsburg, ii, 12–32.
(2) BACKHAUS AND APPEL, O. 1900 Production of aseptic milk. Ber. Landw. Inst. Univ. Konigsburg, v, 73–102.
(3) BREED, A. F. 1928 Micrococci present in the normal cow's udder. N. Y. (Geneva) Agri. Exp. Sta. Tech. Bul. 132.
(4) COPELAND AND OLSEN, T. M.: 1929 The bacterial flora of normal cow's udders. S. Dakota Agri. Exp. Sta. Bul. 218.
(5) HARDING, H. A., AND WILSON, J. K. 1923 A study of the udder flora of cows. N. Y. (Geneva) Agri. Exp. Sta. Tech. Bul. 27.
(6) LUX, A. 1903 On the bacterial content of freshly drawn milk. Centrl. f. Bakt., Abt. 2, xi, 267.

(7) ORLA-JENSEN 1921 Dairy Bacteriology, p. 21. P. Blakiston's Son & Co., Philadelphia.
(8) RUSSELL, H. L. 1894 The source of bacterial infection and the relation of the same to the keeping quality of milk. Wis. Exp. Sta. Rept., pp. 150–165.
(9) STOCKING 1906 Conn. (Storrs) Agri. Exp. Sta. 18th. An. Rept., p. 66.
(10) TROUT, G. M. 1929 Michigan Agri. Exp. Sta., ii, no. 4, 172.
(11) VON FREUDENREICH 1904 Centrl. f. Bakt., 2 abt., xiii, 281.

THE EFFECT OF LIPINS ON THE FAT TEST OF BUTTERMILK*

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INTRODUCTION

As early as 1897 Storch (10) recognized differences in the fat percentage of buttermilk when different testing methods were used. He found that the Rose method gave results 0.04 per cent higher for milk and 0.15 per cent higher for buttermilk than was obtained by drying the samples with kaolin powder and extracting with a Soxhlet apparatus. Mitchell (6) found that buttermilks tested 0.1 to 0.2 per cent by the Babcock method; 0.3 to 0.45 per cent by the rigorous Babcock test and 0.45 to 0.60 by the Roese-Gottlieb method. Lecithin materials have been shown to be present in milk and milk products. Dornic and Daire (3) report 0.0332 per cent of lecithin in buttermilk. Thurston and Peterson (11) working with synthetic milks, to which fat and lecithin were added, concluded that the major portion of the difference between the Babcock and the gravimetric methods of analysis was due to the failure of lecithin to show as fat in the Babcock method. Chapman (1) gives 20.25 as the percentage of the ether extract (in the Mojonnier analysis) that is lecithin. This is equivalent to 0.1302 per cent of the weight of the buttermilk. He further states that when synthetic milks such as were used by Thurston and Peterson (11) were used by him, the percentages of total lecithin added which affected the Babcock, the butyl alcohol and the Mojonnier analyses were respectively 71.0, 68.5 and 76.5 per cent.

Discrepancies among the various methods of analysis of buttermilks do exist. The work so far reported on the effect of lecithin in buttermilk testing has been done on synthetic milks, with the

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exception of Chapman's (1) analyses of the Mojonnier extracts of several dairy products. It is the purpose of this paper to investigate the extent to which lipins affect the Roese-Gottlieb method of analyzing buttermilk and to offer a tentative hypothesis for discrepancies among the tests not accounted for by lipin. The material herein presented is but a preliminary report of a study of the nature of the fatty materials found in buttermilk.

EXPERIMENTAL

Investigation of extraction methods

In order to avoid errors inherent with small quantities of materials, various extraction methods were investigated. All methods which involved drying the buttermilk before extraction gave weights which accounted for but part of the fatty material extracted by the Roese-Gottlieb method. An analysis of these extracts gave the percentage of the weight of the buttermilk that was lecithin from 0.005 to 0.0330 per cent. Continuous wet extraction methods emulsified to so great an extent as to be useless.

The Roese-Gottlieb method, as applied by Thurston and Peterson (11) to 100-gram samples of buttermilk, was finally adopted, with slight modification. In brief the method used was as follows:

One-hundred-gram samples of buttermilk were weighed into 150-cc. beakers. These samples were transferred to 1-liter separatory funnels. The beakers were rinsed successively with each of the reagents, subsequently added, to insure quantitative transfer; 20 cc. of distilled water followed by 20 cc. concentrated NH_4OH were added and the mixture was shaken thirty seconds; 100 cc. ethyl alcohol were added and the mixture was again shaken thirty seconds; 200 cc. ethyl ether and 200 cc. petroleum ether were added in the order given. The mixture was shaken one minute after the addition of each of these reagents. When the emulsion had broken, the lower layer was run into a beaker. The ether-fat layer was run into an 800-cc. beaker. The buttermilk-water-ammonia-alcohol portion was then returned to the liter separatory funnel. One-hundred-cubic-centimeter por-

tions of ether and petroleum ether were added successively and the mixture was shaken thirty seconds after the addition of each reagent. The ether-fat layer from this extraction was combined with the first extract in the 800-cc. beaker. A pinch of powdered pumice was added to the beaker and the ethers were driven off over a steam hot plate until about 30 or 40 cc. of materials remained. This residue was then transferred through a dry filter into either a fat extraction flask, to determine the weight of the extract, or a

TABLE 1

A comparison of the percentage of fatty materials by the Macro-Roese-Gottlieb, the Roese-Gottlieb, and the butyl alcohol analyses

SAMPLE NUMBER	MACRO-ROESE-GOTTLIEB METHOD	ROESE-GOTTLIEB METHOD	BUTYL ALCOHOL METHOD
	per cent	*per cent*	*per cent*
1	0.62	0.650	0.64
2	0.58	0.636	0.57
3	0.68	0.670	0.72
4	0.64	0.660	0.66
5	0.64	0.610	0.59
6	0.71	0.710	0.74
7	0.78	0.668	0.71
8	0.59	0.600	0.54
9	0.71	0.657	0.61
10	0.77	0.790	0.78
11	0.67	0.670	0.68
12	0.77	0.820	0.76
13	0.77	0.770	0.74
14	0.69	0.690	0.66
15	0.76	0.790	0.62

Kjeldahl flask, for digestion of the extract for the phosphorus analysis to determine the lipin content, or to an iodine flask, for the determination of the iodine absorption value of the extract.

The above method of extraction will be referred to throughout this paper as the Macro Roese-Gottlieb method of analysis. That it is representative of the official method is shown in table 1 which gives a comparison of the "fat" percentage by this method, the Macro Roese-Gottlieb method, and the butyl alcohol method of analysis.

Lecithin which affects the Roese-Gottlieb Fat Analysis of Buttermilk

The fatty extract from 100-gram samples of buttermilk was transferred to 500 cc. Kjeldahl flasks, in petroleum ether solution. The petroleum ether was evaporated and the samples were digested and analyzed by Koch and Woods (4) modification of

TABLE 2

The percentage of distearyl lecithin in buttermilk and the fatty extract of buttermilk

Fatty materials for analysis obtained by the Macro-Roese-Gottlieb extraction.

SAMPLE NUMBER	WEIGHT OF EXTRACT	WEIGHT. DISTEARYL LECITHIN	DISTEARYL LECITHIN IN EXTRACT	DISTEARYL LECITHIN IN BUTTERMILK
	grams	*grams*	*per cent*	*per cent*
1	0.6177	0.1537	25.01	0.154
2	0.5754	0.1475	25.64	0.148
3	0.6701	0.1423	21.83	0.142
4	0.6342	0.1595	25.16	0.159
5	0.6362	0.1406	22.79	0.141
6	0.7066	0.1279	18.54	0.128
7	0.7812	0.1762	22.56	0.176
8	0.6030	0.1482	25.00	0.148
9	0.7066	0.1663	23.53	0.166
10	0.7721	0.1603	20.77	0.160
11	0.6749	0.1491	22.09	0.149
12	0.8240	0.1577	21.30	0.158
13	0.7680	0.1467	19.09	0.147
14	0.6901	0.1028	14.89	0.103
15	0.7573	0.1485	18.73	0.149
Average			21.79	0.149

Neuman's (7) wet digestion method. The phosphorus was precipitated as ammonium phosphomolybdate, dissolved in standard sodium hydroxide, boiled to expel ammonia and titrated with standard hydrochloric acid.

The equation of the reaction under the conditions described is, according to Neuman (7):

$$(2(NH_4)_3PO_4) \cdot (24\ MoO_3) \cdot (4\ HNO_3) + 56\ NaOH \rightarrow$$
$$24\ Na_2MoO_4 + 4\ NaNO_3 + 2\ Na_2HPO_4 + 32\ H_2O + 6\ NH_3$$

Therefore, one molecule of P_2O_5 or of $Mg_2P_2O_7$ is equivalent to 56 NaOH. From this ratio the distearyl lecithin equivalent to 1 cc. N/1 NaOH can be calculated by the following formula:

$$0.04 \times \frac{Mg_2P_2O_7}{56\ NaOH} \times 7.27 = 0.02891 \text{ gram distearyl lecithin equivalent to 1 cc. N/1 NaOH}$$

The results of these analyses are presented in table 2.

The effect of sulfonation of fatty materials in the Babcock test

When the foregoing analyses did not show that the discrepancies among the tests, generally used for buttermilk analysis, were due wholly to lipin bodies, an explanation was sought for that portion of the error not due to lipins. It was considered that, since the fat particles in buttermilk were of extremely small size, the sulfuric acid used in the test would have a large surface for reaction with these fats.

It is well known that sulfonated oils are widely used in the textile industry. Many explanations for the reactions of sulfonation have been offered. Pomerang (8) offers an explanation which, in view of the fact that the rigorous Babcock method accounts for a greater part of the fatty materials of buttermilk than does the regular Babcock method, seems best fitted to the present discussion. He believes that no formula can be offered for the reaction product between the fixed oils and sulfuric acid, but that it is definitely of colloidal nature, and that the reactions are of absorptive nature. Radcliffe and Medofski (9), comparing the constants of the acids of oils before and after sulfonation found in practically all cases, that after sulfonation the iodine values were lower, indicating increased saturation, that the saponification number of the sulfonated oil increases, indicating lactone formation, and that the acetyl values were higher, indicating the formation of hydroxy acids. These authors state that the ease of sulfonation depends not on the degree of unsaturation of the oil but rather on the percentage of olein or oleic acid present. Crowe (2) states that the higher the degree of sulfonation in an oil the greater the solubility of the product in water.

Butterfat contains a high percentage of olein. If the greater part of the sulfonation took place with the unsaturated fats, and caused them to become soluble or to assume a colloidal state which defied centrifugal separation under the usual methods employed in the Babcock test, it seemed logical to investigate the fatty materials of buttermilk to determine whether sulfonation of the olein accounted for that portion of the discrepancy be-

TABLE 3

Relation between the calculated weight of saturated fat (weight of fatty material by gravimetric method less the sum of the lipins, sterols and olein) and the measured Babcock analysis

SAMPLE NUMBER	WEIGHT OF EXTRACT (FROM ROESE-GOTTLIEB ANALYSES)	WEIGHT OF LIPIN IN EXTRACT (AS DI-STEARYL LECITHIN)	WEIGHT OF STEROLS (ASSUMED)	WEIGHT OF OLEIN (CALCULATED)	WEIGHT OF SATURATED FAT (CALCULATED)	BABCOCK TEST	CALCULATED SATURATED FAT LESS MEASURED BABCOCK TEST
	grams	*grams*	*grams*	*grams*	*grams*	*per cent*	
1	0.670	0.1503	0.02	0.2171	0.220	0.17	0.050
2	0.670	0.1422	0.02	0.2826	0.225	0.17	0.055
3	0.660	0.1595	0.02	0.2054	0.275	0.21	0.065
4	0.610	0.1447	0.02	0.1881	0.257	0.15	0.107
5	0.710	0.1280	0.02	0.3520	0.210	0.17	0.040
6	0.668	0.1762	0.02	0.2815	0.190	0.20	−0.010
7	0.600	0.1485	0.02	0.2046	0.230	0.34	−0.110
8	0.657	0.1663	0.02	0.1735	0.297	0.32	−0.023
9	0.790	0.1603	0.02	0.2404	0.369	0.41	−0.044
10	0.670	0.1491	0.02	0.1876	0.313	0.28	0.033
11	0.820	0.1577	0.02	0.3483	0.294	0.21	0.084
12	0.770	0.1467	0.02	0.3276	0.256	0.20	0.076
13	0.690	0.1028	0.02	0.2882	0.279	0.13	0.149
14	0.790	0.1485	0.02	0.3363	0.285	0.19	0.095
15	0.730	0.1172	0.02	0.3916	0.201	0.16	0.041

tween the Babcock and the gravimetric methods, not shown by the lipin content. Therefore, the following experiment was undertaken.

Two samples (100 grams each) from each lot of buttermilk analyzed, were extracted as previously described. On one of these extracts the usual Hanus iodine absorption method was used to determine the total weight of iodine, which the extract would absorb. With the other sample, the weight of the extract and the

lipin content (as distearyl licithin) were determined. Four assumptions were then made, one, that the lipin material had one double bond per molecule (Maclean (5)); two, that the unsaturated fats were present as olein, which did not allow for any mixed fats; three, that buttermilk contained 0.02 per cent by weight of sterols (as cholesterol), since the average of the value reported by Thurston and Peterson (11) was 0.0178; and, fourth, that the weight of fatty extract, determined by the gravimetric method, less the sum of the weights of lipins, sterols, and olein, was saturated fat. The iodine equivalent to the sterol and lipin content was calculated. This weight of iodine was subtracted from the total iodine abosrbed. From this difference the weight of olein was calculated. In the manner indicated above the weight of saturated fat was calculated. It was considered that the regular Babcock method accounted for saturated fat only, whereas the butyl alcohol and the gravimetric methods measured saturated fats, unsaturated fats, lipin and sterols. To what extent this hypothesis is substantiated by the experimental data is shown in table 3.

In the above calculations the molecular weight of lecithin was taken as 777.71; the gravimetric factor for iodine equivalent to lipin was $\frac{I_2}{\text{Mol. wt. lecithin}}$; that for iodine equivalent to sterols was $\frac{I_2}{\text{Mol. wt. cholesterol}}$ and that for olein equivalent to iodine was $\frac{\text{Mol. wt. olein}}{3\ I_2}$.

SUMMARY AND CONCLUSIONS

When buttermilk is dried before it is extracted the lipin material seems to be destroyed or at least it is not wholly extracted by ether or by petroleum ether from the dried product. This fact, together with the fact that the average of the lipin analyses reported is 0.149 per cent appears to account for the 0.15 per cent higher fat percentage by the Rose method, than by the Soxhlet method on dried buttermilk which was reported by Storch (10).

The Macro Roese-Gottlieb extraction method used throughout

this work gave weights of fatty material which are in agreement with the Roese-Gottlieb analyses of the buttermilk.

Lipin materials were found in the extracts in quantities ranging from 14.89 to 25.64 per cent of the weight of the extract, with an average of the values 21.79 per cent. The percentages of the weight of the buttermilk that was lipin ranged from 0.103 to 0.176 per cent, with an average of the values 0.149 per cent. These figures are in close agreement with the values reported by Chapman (1), which were 20.25 per cent of the weight of the extract and 0.1302 per cent of the weight of the buttermilk.

The data presented in table 3 would indicate that sulfonation of fats does occur in the Babcock test. They seem to indicate likewise that the error due to sulfonation is at least double that due to lipin materials.

REFERENCES

(1) Chapman, O. W.: The effect of lecithn in dairy products upon the butterfat determination. Jour. Dairy Sci., 1928, xi, 429–435.

(2) Crowe, J. B.: Sulphonated oils. Amer. Dyestuffs Reporter, 1926, xv, no. 10, 379–382. Reported in Chem. Abs., 1926, xx, 2589 as Am. Dyestuff Rept., xiv, 379–82 (1926) as a result of a typographical error in Amer. Dyestuffs Reporter.

(3) Dornic and Daire: Contribution a'l'etude de le lecithin du lait. Ann. Fals., 1910, xxx, 533–538; Rev. General du Lait, 1910, viii, 385–394.

(4) Kock, W., and Woods, H. S.: The quantitative estimation of the lecithins. Jour. Biol. Chem., 1906, i, 203–211.

(5) Maclean, H.: Lecithin and Allied Substances. The Lipins, p. 28. Longmans, Green & Co., London, 1918.

(6) Mitchell, J. W.: Churning losses. Chicago Dairy Produce, 1921, xxviii, no. 31, 16–19.

(7) Neuman, A.: Einfache Veraschunge-methode (Sauregemisch-Veraschung) und Vereinfachte Bestimmungen von Eisen, Phophorsaure, Salzsaure, und anderen Aschenbestandtheilem unter Benutzung deiser Sauregemisch-Veraschung. Zeit. Physiol. Chem., 1902, xxxvii, 115–142.

(8) Pomerang: Ueber Sulfurierung von Oelen allgemein und besonders von Fischölen. Chem. Umschau, 1923, xxx, 133–134.

(9) Radcliffe, L. G., and Medofski, S.: Jour. Soc. Dyers Colourists, 1918, xxxiv, 23–35.

(10) Storch, V.: The structure of fat globules in cow's milk. The Analyst, 1897, xxii, 196–207.

(11) Thurston, L. M., and Peterson, W. E.: Lipins and sterols as sources of error in the estimation of fat in buttermilk by ether extraction methods. Jour. Dairy Sci., 1928, xi, 270–283.

AIR IN BUTTER*

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The presence of air in butter may have a definite relation to the quality of this product. The variableness of air in butter probably has a direct correlation to weight in making it into prints, for butter is cut into prints by volume.

According to Pickerill and Guthrie (2), the amount of air in butter averages approximately 4 per cent. This agrees with Rahn and Mohr (3), although the range given by Pickerill and Guthrie is wider, varying from 0.5 to 6.0 per cent in one table to 4 to 14.9 per cent in another in which the butter was older.

Recently the author has made several determinations of the amount of air in butter, in a study of the effect of working on the percentage of air. These data will be presented later in this article.

THE METHOD OF DETERMINING THE VOLUME OF AIR IN BUTTER

The amount of air, in the butter in this study, was determined in general by the method of Rahn and Mohr (3). The basis of this method of ascertaining the amount of air is the fact that air is the only component of butter that expands under vacuum. The expansion under vacuum, therefore, is measured and then the amount of air that causes this expansion is calculated by the law of Boyle-Mariotte (1).

One modification on the equipment was made, and one change in the procedure was advantageous. The equipment that was employed in this study is shown in figures 1 and 2. The modification in equipment consisted of the addition of the curved tube in the cap of the glass cylinder. The purpose of this curved tube is to prevent the milkfat that escapes from the metal cone below,

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from entering the graduated tube and interfering with accurate reading.

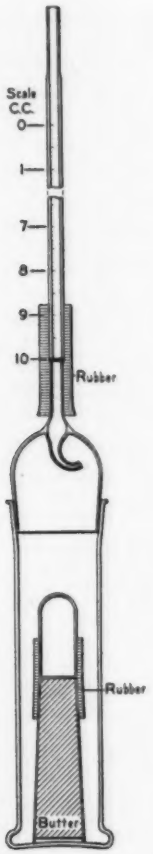

FIG. 1. THE CYLINDER, THE CONE OF BUTTER, AND THE GRADUATED TUBE IN WHICH THE AIR EXPANSION IS READ

The change in the method is to push the butter up in the metal cone at least $\frac{1}{16}$ of an inch from the lower edge as shown in figure 1. This is accomplished by covering a cork stopper that was approximately the size of the opening in the base of the cone,

with wet parchment paper. The purpose of the wet parchment is to prevent the sticking of the butter to the stopper. A little

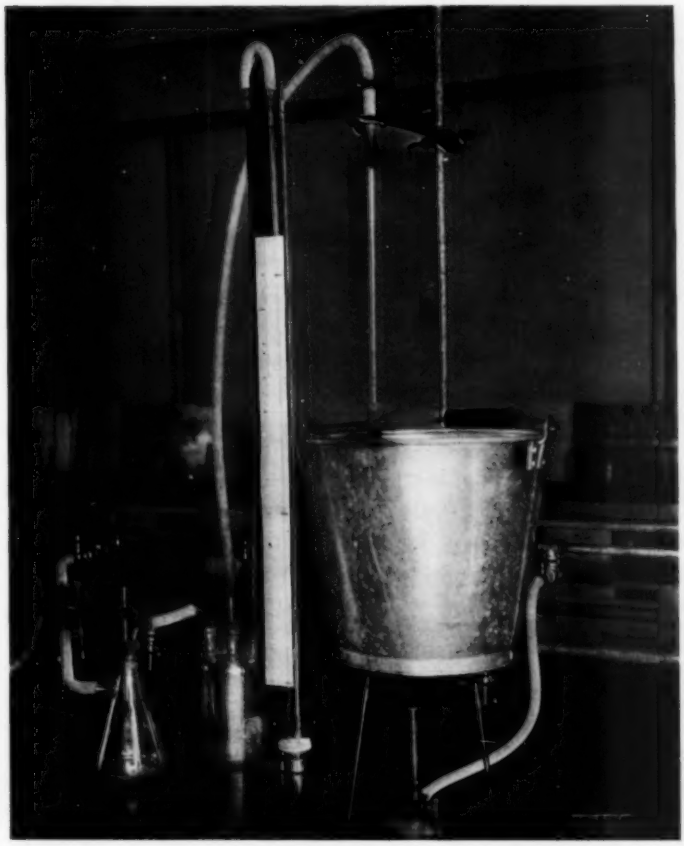

FIG. 2. THE COMPLETE APPARATUS SHOWING THE VACUUM EQUIPMENT; THE EQUIPMENT IN FIGURE 1 IS IN THE PAIL

pressure is sufficient to force the butter $\frac{1}{16}$ of an inch up the cone. The purpose of forcing the butter a little way from the lower edge of the cone is to retain practically all of the milkfat in the

cone, so that only a little of it would flow from under the cone and accumulate in the top of the cylinder.

The cylinder is 4.5 cm. in diameter and 20 cm. long. The dome of the top extends 4 cm. above the main section of the cylinder and the connecting nipple adds about 3 cm. to the entire length of the cylinder, making the over-all length 27 cm.

The cones were obtained from a local tinner and were made of tinned iron. Those of the first set were too narrow at the top, for it was almost impossible to fill them by simply pressing them into the butter. The second set of cones which were filled easily, were 8.2 cm. long, 2.8 cm. in diameter at the base and 2.1 cm. in diameter at the top.

Figure 1 shows the cylinder in which the sample of butter is placed and to which the vacuum is applied. Figure 2 shows the complete apparatus but only the vacuum apparatus is visible because the cylinder, figure 1, is in the pail. Attached to it is the glass tube graduated in 0.1 cc. on which the volume readings are made.

The graduated tube is connected with the glass jar which acts as a trap. The tube containing the mercury is also attached to this trap. It, in turn, is connected with the air line through another trap. The two petcocks which aid in the fine control of the apparatus are directly connected with the latter trap. The centimeter scale on the cardboard is sufficiently large to be easily read. The steps in making the determination are as follows:

1. The metal cones are dried and weighed.

2. The cones are filled by pressing them into a one-pound print of butter. The most satisfactory procedure in filling is to force the cone clear through the print, as it rests, crosswise, on the table. It is necessary that both the top and bottom surfaces of the butter be smooth so that air will not collect in the apertures.

3. The butter is forced at least $\frac{1}{16}$ of an inch up the cone as described above.

4. The metal cones containing the butter, are wiped dry with a clean cloth and then weighed.

5. The glass head, made from a heavy glass test tube, is attached to the small end of the metal cone with a heavy rubber

tube. This connection is made under the surface of cold water from which the air has been previously driven out by boiling. The temperature of this water must be sufficiently low in temperature to prevent the melting of the butter.

6. The cone with the glass head attached was slipped into the cylinder as shown in figure 1. This is accomplished under the surface of the water in the pail which may be seen in figure 2. This water must have been previously boiled to expell the air.[1] Then the other connections, as seen in figure 2, were consummated as carefully and as quickly as possible. In order that no air be caught in the base of the cone, it is essential that the cone be entered in the cylinder in the horizontal position. The pail, therefore, must be sufficiently large for the cylinder to extend across it with room to spare. The vacuum was obtained through an air line connected with a Mojonnier tester.

7. In this study, the temperature in the pail ranged from 39° to 49°C. The exact temperature was always recorded with each set of readings. A vacuum of 25 mm. was held for five minutes during which time all of the milkfat melted. A ten-minute period is a little more satisfactory.

8. The first reading on a single cone of butter was always made at 760 mm. (mercury) pressure. The reading was obtained from the tube as seen in figure 1, which was graduated in 0.1 cc. The succeeding four or five readings were made at steps of decreasing pressures, as read on the mercury manometer. For illustration the following is a set of readings:

Reading in cc., fig. 1 or 2.....	4.1	3.8	3.65	3.15	2.40	1.00
Reading in mm., fig. 2.	760	650	600	500	400	300

9. After the readings in step 8 were taken the vacuum was gradually released and then another set of readings were made on the same cone of butter. The arrangement of the scale on the gas

[1] If the water in the pail is used too long, small bubbles of air may be observed floating to the top of the cylinder when the vacuum is on. This is an indication that the water should be reboiled.

burette is such that an expansion of the air in the butter, corresponds to a lower scale reading on the burette. The following data show the readings on this same illustrative determination:

Readings in cc., figs. 1 or 2.....	3.90	3.60	3.20	2.60	1.65
Readings in mm., fig. 2.....	760	650	550	450	350

10. The Boyle-Mariotte law (1) operates according to the equation $V_0 P_0 = V_1 P_1$.

V_0 is the unknown volume X at P_0 or atmospheric pressure of 760 mm.

TABLE 1

Calculating the volume of air in butter

$760 - P$	P	READING IN GRADUATED TUBE	Dv	X
	760	4.1		
110	650	3.8	0.3	1.772
160	600	3.65	0.45	1.687
260	500	3.15	0.95	1.826
360	400	2.40	1.70	1.888
460	300	1.00	3.10	2.021
Average.....				1.839

V_1 is the volume at the decreased pressure P_1

$V_1 = X +$ the measured increase of the volume or

$V_1 = X + dv$

$$X \cdot 760 = (X + dv) P_1$$

$$X \cdot 760 = XP_1 + dv P_1$$

$$X (760 - P_1) = dv P_1$$

$$X = \frac{dv P_1}{760 - P_1}$$

The application of these equations are made directly in table 1, to data in this study. In this table the heading "$760 - P$" is 760 mm., atmospheric pressure minus the pressure at which the reading was taken. "P" is the pressure of the air in the butter,

at which the reading was made. "Reading in graduated tube" is the reading on the tube, as seen in figure 1. "*Dv*" is the difference between the reading on the graduated tube at 760 mm. and the one immediately concerned. "*X*" is the unknown volume at atmospheric pressure of 760 mm.

11. The next calculation is to correct for temperatures. The air content of butter is always measured at melting temperatures. The volume occupied by water vapor at the various temperatures must be considered. The saturated pressure of water vapor at 40°C. is 55 mm. mercury or 55/760 of the air space calculated for 760 mm. or atmospheric pressure.

TABLE 2

Calculating factors for different temperatures

VAPOR PRESSURE	TEMPERATURE	FACTOR	VAPOR PRESSURE	TEMPERATURE	FACTOR
mm.	°C.		mm.	°C.	
49	38	0.875	71	45	0.848
52	39	0.872	75	46	0.843
55	40	0.868	79	47	0.838
58	41	0.864	83	48	0.833
61	42	0.861	87	49	0.829
64	43	0.858	92	50	0.823
68	44	0.852			

As an example of calculation for 40°C.

$$V_{40} = \frac{X\ (760 - 55)}{760} = \frac{X\ 705}{760}$$

The volume was further corrected to a standard temperature of 40°C.

$$V_{20} = V_t \frac{273 + 20}{273 + t}$$

The following is an example of changing the volume at 40°C. to the volume at 20°C.

$$V_{20} = V_{40} \frac{273 + 20}{273 + 40} = \frac{X\ 705 \cdot 273 + 20}{760 \cdot 273 + 40} = X\ 0.868$$

This corrective factor, 0.868, must be applied to the average reading in column "X" of table 1 to bring 40°C. to the saturated-pressure of 20°C. The following table contains the corrective factor and vapor-pressure for the different temperatures.

TABLE 3

The effect of working butter on the percentage of incorporated air

SAMPLE	PRINT 1		PRINT 3		PRINT 5		PRINT 7		PRINT 9		PRINT 11	
	First reading	Second reading	First reading	Second reading	First reading	Second reading	First reading	Second reading	First reading	Second reading	First reading	Second reading
Section 1:												
a	6.008	5.453	4.552	4.991	3.287	3.059	3.522	3.600	3.547	4.602	5.635	5.718
b	6.660	6.671	4.313	4.674	2.181	2.382	3.658	3.835	5.328	5.626	5.176	5.399
c	7.026	6.860	6.450	6.383	2.579	2.509	4.186	4.118	4.965	5.560	5.432	6.274
d	6.168	6.660	4.169	4.332	4.590	4.307	4.272	4.161	5.005	4.612	4.516	4.654
e	6.648	6.351	3.260	3.633	2.797	2.392	3.658	3.731	4.887	5.149	5.122	5.302
f	6.021	5.710	4.214	4.533	2.343	2.476	4.042	4.064	5.065	5.242	4.140	4.429
Average	6.421	6.284	4.493	4.758	2.962	2.854	3.889	3.918	4.799	5.131	5.003	5.295
	PRINT 2		PRINT 4		PRINT 6		PRINT 8		PRINT 10		PRINT 12	
Section 2:												
a	7.390	7.081	5.307	5.002	5.111	5.368	4.932	4.932	4.636	4.630	5.242	5.592
b	6.713	6.602	5.578	6.008	5.151	5.125	2.846	3.887	3.801	4.196	5.063	5.029
c	3.615	3.595	4.188	4.612	4.844	4.780	4.384	4.586	4.348	4.381	5.820	6.314
d	6.310	6.595	6.348	6.221	5.966	5.212	5.765	5.718	2.603	2.912	6.301	6.317
e	6.260	6.533	4.877	4.944	6.193	5.904	5.735	5.584	3.971	4.077	6.076	6.395
f			4.392	4.269	6.090	5.793	5.246	4.882	4.115	3.929	6.008	6.240
Average	6.668	6.703	5.115	5.176	5.559	5.363	4.817	4.931	4.174	4.243	5.752	5.981

The grand average of the fairly well worked butter of section 1 was 4.651 per cent air, while the grand average of the thoroughly worked butter of section 2, was 5.374 per cent air. This is a difference of 0.723 per cent.

12. The final computation of the percentage of air in butter follows this equation,

$$\frac{X \cdot F \cdot 100}{Wt} \cdot X = 1.839$$

as noted in table 1. F = 0.852 which is the temperature correction factor at 44°F., as seen in table 2. The 100 is used to con-

vert the quotient into percentage. And Wt = the weight of the butter which was 36.1542 grams.

$$\frac{1.839 \cdot 0.852 \cdot 100}{36.1542} = 4.333 \text{ per cent}$$

In like manner the calculation of the volume of air in terms of percentage may be obtained from the second set of readings which are recorded in step 9.

THE RELATION OF THE AMOUNT OF WORKING THAT BUTTER RECEIVES TO THE AMOUNT OF INCORPORATED AIR

The data in this study of the relation of the amount of working that butter receives to the mount of air incorporated in it, may be seen in table 3. The first section of this table gives figures on butter that was worked sufficiently to prevent the formation of mottles. This butter contained fairly large droplets of incorporated water. The second section presents figures on the same lots of butter that were thoroughly worked. This latter butter appeared dry, for the moisture droplets were very small.

The butter was made in a combined churn and worked in 6 different churnings. The prints, weighing 1 pound, were taken in the following manner; print number 1, was obtained from the middle of the churn when the butter was fairly well worked; print number 2, was taken from the same location in the same churning after the butter was thoroughly worked. In like manner, the remaining pairs of prints were obtained from the churn when the butter was fairly well worked, and when it was thoroughly worked.

The samples (a, b, c, d, e, f) are the conefuls that were taken from each print. The columns that are headed "First reading" or "Second reading" give data on the first series of readings after the vacuum was applied; and then on the second series after the vacuum was released, and again applied to the same sample of butter. Each single reading for each sample of butter is an average of 5 or 6 individual sets of figures. Both readings of sample c in print 2, and sample d in print 10 were omitted for they were much out of line.

Table 3 shows an average increase of air content in the butter, due to working of 0.701 per cent. The lowest increase was 0.227 per cent as found in the first readings of prints 1 and 2. The highest increase was 2.521 per cent as seen in the first readings of prints 5 and 6. Prints 9 and 10 reserved the figures and gave a decided decrease even though the two readings of sample d were omitted.

These data which show a little greater variation than that found indicate that one effect by Rogers (4), of working butter is to slighly increase the percentage of incorporated air. Under certain conditions, the variation in air content between the butter that is fairly well worked and that which is thoroughly worked, may be sufficient to materially affect the procedure of packing butter, particularly of printing it; for print butter is packed by volume and sold by weight.

REFERENCES

(1) CLARKE, F. W., AND DENNIS, L. M.: Law of Boyle-Mariotte. Elementary chemistry, 1902, p. 71.

(2) PICKERILL, H. M., AND GUTHRIE, E. S.: Two factors causing variation in the weight of print butter. Cornell Univ. Agri. Exp. Sta. Bul. 355, 98–112 (1915).

(3) RAHN, OTTO, AND MOHR, WALTER: Der Luftgehalt der Butter. Milch. Forsch., 1924, i, 213–221.

(4) ROGERS, L. A.: Fishy flavor in butter. U. S. Agr. Dept., An. Indus. Bur. Cir. 146, 1–20 (1909).

A NEW FORM OF LACTOSE CRYSTAL FOUND IN SANDY ICE CREAM*

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In contrast with the lactose crystals usually found in sandy ice cream, originally described by Zoller and Williams (1) as lactose hydrate ($C_{12}H_{22}O_{11} \cdot H_2O$) and characterized by a distinct tomahawk appearance, another form of crystalline milk sugar has been found in an investigation conducted in these laboratories. This type of crystal is diamond in shape and may develop in ice cream when produced and held under certain conditions.

These crystals which form at a slow rate, and apparently from only very viscous solutions, have likewise been proven to be lactose hydrate.

Since this type of crystal had not heretofore been observed in ice cream, there was some doubt with respect to its preliminary identification; its unusual shape suggested the possibilities that it might be either the beta (β) form of lactose, or sucrose in the early stage of development.

The new crystals were first examined crystallographically and later chemical and other physical tests for their identification were made.

DETECTION OF NEW FORM OF CRYSTAL IN ICE CREAM

The diamond shaped crystal was first found in ice cream that contained 12 per cent butterfat, 16 per cent sugar, 12 per cent serum solids, and 0.3 per cent gelatin. The unusual shape of this crystal was discovered while making a microscopic and photographic study of grease covered slides (fig. 1), designed by the authors for the purpose of determining and recording the degree of texture variations occurring in ice cream. The ice cream had been held in the hardening room about five weeks

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when the sandy texture was first observed. The "sand" had developed in three cans of ice cream and after the new form of crystal had been noticed it was possible to separate a relatively large quantity for chemical and microscopic study. A considerable portion of these crystals has been saved for future reference.

SEPARATION AND IDENTIFICATION

The crystals were removed from the ice cream by first centrifuging the melted product in large glass tubes. The fat and other liquid were then removed by decantation and the crystals obtained (fig. 2) were washed by shaking with a slightly ammoniacal solution of equal parts of alcohol and water which had been cooled to −5°C., the solution being made slightly alkaline in order to dissolve the precipitated and denatured proteins. This procedure was repeated several times to insure the complete removal of the protein from the surface of the crystals and, after a final washing with ether, clean crystals were obtained (fig. 3).

The crystals were dried at room temperatures and were submitted to Dr. Edgar T. Wherry of the Bureau of Chemistry and Soils for identification. These crystals proved to be too small to permit a full description of their crystallographic characteristics; furthermore they could not be compared with all possible forms of lactose since at that time there appeared to be no information in the literature concerning the crystallography and optical properties of β-lactose (2). It was necessary therefore to prepare several samples of this type of crystal (fig. 4) for more complete microscopic measurements and for chemical examination.

A preliminary crystallographic examination showed that these diamond shaped crystals had the refractometric characteristics of alpha (α) lactose hydrate.

CHEMICAL AND PHYSICAL EXAMINATION

The reduction test with Fehling's solution proved conclusively that the new crystals were not sucrose; this fact and their occurrence in a relatively large quantity showed that they were probably some form of lactose.

A series of melting point determinations of the diamond shaped

crystals were made and compared with this property of α-lactose hydrate and β-lactose anhydride. The results of these tests are shown in table 1.

The melting point determinations, made upon three separate samples of β-lactose anhydride crystals prepared in these laboratories, were in reasonable agreement with the melting point given by Gillis; and, melting point determinations made on three

TABLE 1

Melting point determinations of lactose and sucrose crystals

	MELTING POINT*
	°C.
β-lactose anhydride:	
Leighton, prepared by	252.9
Williams, prepared by	252.4
Williams, (large crystals)	253.4
Gillis (3)	252.2
α-lactose hydrate:	
Diamond shaped crystals	199.3
Diamond shaped crystals	201.1
Diamond shaped crystals	199.8
Gillis (3)	201.6
α-lactose anhydride:	
Gillis (3)	222.8
Sucrose (different observers)	160–180

* Melting point tubes containing the sample were immersed beside the thermometer bulb for 5 seconds. The bath used was a $NaNO_3$-K_2CO_3 fusion mixture. Anschutz thermometers (Bureau of Standards calibrated) were used.

samples of the diamond shaped crystals agreed closely with the melting point given for the α-hydrate. These results proved conclusively (*a*) that the new crystals were not β-anhydride or α-anhydride lactose, and (*b*) that they were α-lactose hydrate but a crystal form which heretofore had not been found in sandy ice cream.

The melting points of α-anhydride lactose and sucrose are also shown in the table.

CONDITIONS CAUSING FORMATION OF NEW CRYSTAL FORM OF LACTOSE

The formation of the ordinary type of "sand" (fig. 6) in ice cream is usually attributed to one or more of the following factors: (*a*) a high concentration of lactose, (*b*) insufficiently rapid freezing, (*c*) temperature fluctuations in hardening rooms and

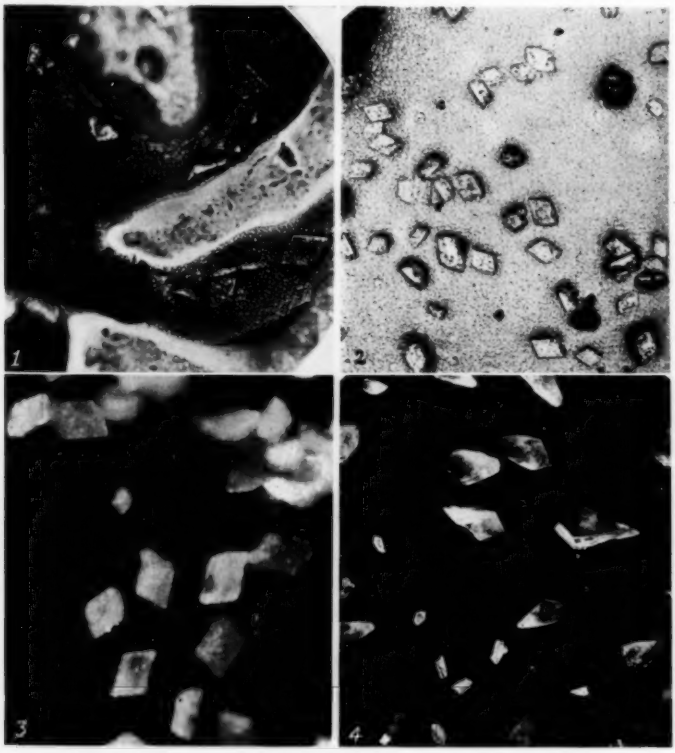

FIG. 1. DIAMOND SHAPED CRYSTALS IMBEDDED IN GREASE COVERED SLIDE

FIG. 2. DIAMOND SHAPED CRYSTALS WITH COATING OF PROTEIN MATERIAL

FIG. 3. DIAMOND SHAPED CRYSTALS WASHED FREE OF PROTEIN

FIG. 4. β-LACTOSE ANHYDRIDE CRYSTALS

(Natural size)

cabinets, and (*d*) the presence of crystal and other nuclei such as nuts, fruit seeds, etc.

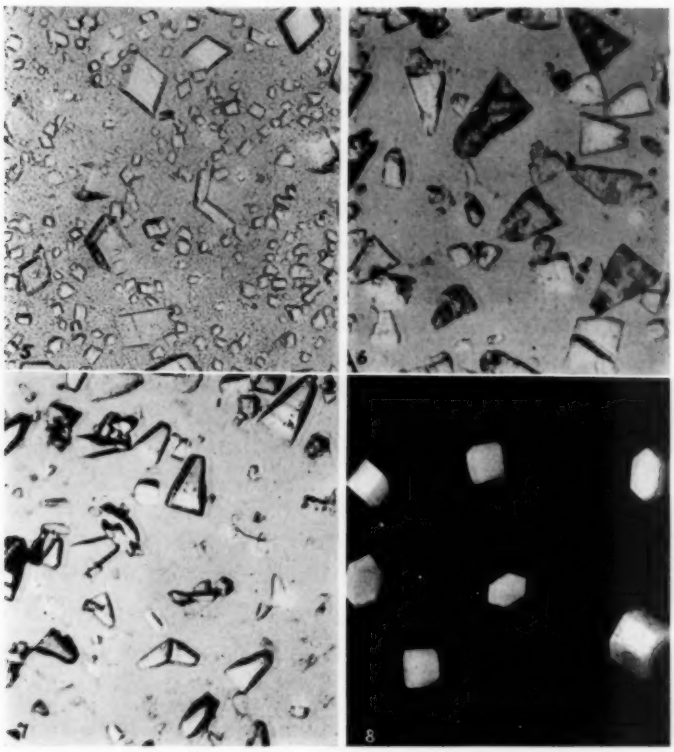

FIG. 5. DIAMOND SHAPED CRYSTALS FOUND IN SANDY SWEETENED CONDENSED MILK. × 130
(Hunziker and Nissen)

FIG. 6. "SAND" CRYSTALS FROM ICE CREAM. × 90
(Zoller and Williams)

FIG. 7. "SAND" CRYSTALS FROM CONDENSED WHEY. × 90
(Zoller and Williams)

FIG. 8. CRYSTALS OF PURE SUCROSE
(Natural size)

The formation of the new type of lactose in ice cream is probably due to the first three mentioned factors and its crystal form, in particular, is likely caused by the high concentration of sucrose.

Thus Hunziker and Nissen (5) have found that while the colloids in milk do not have any material influence on the solubility of lactose, the presence of sucrose does diminish the solubility of milk sugar, and that a high sucrose concentration in lactose solutions also has a very noticeable influence on lactose crystal formation, the shape of the lactose crystal being distinctly modified.

Since these investigators have also shown (4) that "the presence of milk colloids in saturated lactose solutions does in no way interfere with the full development of the lactose crystals" and that diamond shaped crystals are formed in sweetened condensed milk (fig. 5) the occurrence of similarly shaped crystals in ice cream can be attributed to the high concentration of the sucrose in the solution, mainly caused by freezing and separation of a large part of the water. It appears probable that the more viscous the solution from which lactose crystallizes, the more likely is the crystal form to be modified. If, however, the ice cream be maintained at such a high viscosity (frozen hard, or approximately so) as to prevent diffusion, separation of the lactose, if it takes place at all, will result in the formation of minute crystals. Such crystals, or very fine "sand," are practically undetectable when this product is consumed and hence are not objectionable.

SUMMARY

Diamond shaped crystals occurring in "sandy" ice cream have been identified as α-lactose hydrate.

It is believed that the occurrence of this modified form of lactose crystal is caused by the high viscosity of the solution—due in particular to the concentration of the sucrose in the crystallizing medium, which in turn is caused by a freezing out of part of the water.

The relatively large size of the diamond shaped crystals, as well as their quantity, indicates that, for the avoidance of "sand" in ice cream, the viscosity of the solution, in addition to the solubility relationships of lactose, must be considered.

It is recommended that ice cream be kept frozen hard or approximately so, thus preventing diffusion and the growth of relatively large lactose crystals or objectionable "sand."

REFERENCES

(1) Zoller, H. F., and Williams, O. E.: Sandy crystals in ice cream; their separation and identification. Jour. Agr. Research, 1921, xxi, 791–796.
(2) Wherry, E. T.: The crystallography and optical properties of β-lactose. Jour. Washington Acad. Sci., 1928, xviii, 302–304.
(3) Gillis, J.: Rec. trav. chim., 1920, xxxix, 88.
(4) Hunziker, O. F., and Nissen, B. H.: Lactose solubility. Jour. Dairy Sci., 1926, ix, 517–537.
(5) Hunziker, O. F., and Nissen, B. H.: Lactose crystal formation. Jour. Dairy Sci., 1927, x, 139–154.

COTTONSEED MEAL STUDIES

III. HEAVY FEEDING OF COTTONSEED MEAL TO DAIRY CATTLE DURING REPRODUCTION AND LACTATION*

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Cottonseed meal usually furnishes the cheapest available source of a high protein concentrate but it is not heartily recommended by all authorities. It is seldom recommended as a feed for calves and only in small amounts for pregnant or lactating cows. If fed heavily, it is thought to produce injury. Cottonseed meal injury has been attributed to beri-beri, pyrophosphoric acid, bacteria and molds, gossypol, betaine and choline, iron deficiency, protein decomposition products, acidosis, and lack of a factor or factors carried by hay. A lack of a factor or factors carried by hay seems very probable in view of the fact that in sections where cottonseed meal is produced and therefore available, cottonseed hulls have been used largely as a roughage. Cottonseed hulls probably lack a factor or factors carried by a good quality of hay.

The purpose of this investigation was to study the effects of cottonseed meal as the principle source of protein for growth, reproduction, and lactation over a long period of time with a ration otherwise adequate.

This is the third of a series of papers on cottonseed meal to be reported from the Dairy Section of the Michigan Agricultural Experiment Station. It was shown in a previous report (1) that the liberal feeding of cottonseed meal to growing heifers along with timothy hay and corn silage, did not produce the symptoms of cottonseed meal injury. However, when cottonseed meal was fed with wheat straw as a roughage, the symptoms of cottonseed meal injury were manifested.

The present paper is a progress report giving the effect of heavy

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cottonseed meal feeding on health and reproduction of the first generation of dairy cattle to about four years of age.

REVIEW OF LITERATURE

Moore (2) investigated the effect of heavy feeding of cottonseed meal on health and reproduction of dairy cattle. Three lots of 5 animals each were used. The animals in lot I were fed heavily on cottonseed meal with a little other grain and received hay and silage principally as a roughage. Those in lot II received a grain ration containing no cottonseed meal but with cottonseed hulls principally as a roughage, while those in lot III received a good grain ration with hay and silage principally as a roughage, but no cottonseed products.

Milk production and breeding data were given as follows:

	MILK PRODUCTION FIRST 180 WEEKS	TIMES BRED	CALVES DROPPED	MONTHS BETWEEN CALVING	CASES OF GARGET
Lot I	13.7	56	22	14	14
Lot II	14.3	41	24	13	1
Lot III	11.7	29	24	12	2

In lot I, one cow lost two quarters of her udder and two cows lost one quarter each, while in lot II, one cow lost one quarter of her udder. In lot I there were three cases of retained afterbirth, and in lot II one case of abortion. One calf was born dead in lot I, and another was weak.

Moore concluded from these results that the feeding of 5 pounds of cottonseed meal per day for any great length of time is injurious to the dairy cow, causing inflammation of the udder, difficult breeding and retention of the afterbirth, although apparently no symptoms of cottonseed meal poisoning were noted.

Combs and Curtis (3) of the North Carolina Station fed 5 lots of 5 animals each cottonseed meal at the rate of one pound daily per 100 pounds live weight. Cottonseed hulls, beet pulp, and corn silage in various combinations were fed as roughage. Blindness, weakness, abortion, dead and weak calves occurred. Sev-

eral of the animals died, but no deaths occurred in the lot receiving corn silage.

The work of Combs and Curtis has been continued at the North Carolina Experiment Station by them and several other investigators to the present time.

In 1923 (4) these investigators reported that the previous rations were supplemented with 5 to 10 per cent alfalfa meal, with the result that two living calves were prodced and convulsions did not occur.

In 1924 (5) mineral salts added to the ration of cows whose roughage was cottonseed hulls, and grain made up entirely of cottonseed meal, were of no benefit. Calves were born prematurely, and even though some gestation periods were normal, the calves were weak. The addition of small amounts of other supplements which were described in this report as "certain other substances" proved to be highly beneficial. The following statement is made:

> Where cottonseed meal and hulls have been supplemented liberally with corn silage and cracked corn, the cows receiving this ration have not been able to give birth to normal calves and milk normally. But with the addition of small quantities of certain other substances to the rations, these cows have given normal calves and have milked well above the state average.

In 1925 (6) a very interesting fact was mentioned as follows:

> Approximately five years ago twenty head of cattle on heavy cottonseed meal feeding, kept on a lot of four acres of ground, produced apparently normal living calves. In this lot these cows had access to small amounts of grass which they kept clipped short. This herd was later moved to a small closely fenced lot where no green grass was obtained with the astonishing result that abortions, dead and underweight calves, and living blind calves were obtained.

In 1926 (7) four lots of cows which were fed a concentrate ration of from 50 to 100 per cent cottonseed meal with a good roughage reproduced normally. They further reported that during 1926 four groups of 3 animals each were placed on an experiment with wheat straw as roughage and using as concentrates in lot I,

cottonseed meal; lot II, linseed oil meal; lot III, peanut meal, and in lot IV, soy bean meal. All animals received minerals.

In 1927 (8) the roughage was changed in the above lots to beet pulp and cottonseed hulls. All animals in all groups reacted similarly.

One animal out of the first 3 lots and all out of lot IV died. The addition of cod liver oil alleviated the deficiency symptoms of the remaining animals.

In another group of cows, those animals receiving the poorest quality of roughage failed to respond as did those where alfalfa hay was used.

Bell and Williams (9) reported that cottonseed meal fed at the rate of one pound per day to 1000 pounds live weight to mares during the period of pregnancy showed no ill effects from its consumption, nor were any ill effects noticeable on the colts when foaled, nor did the feeding of cottonseed meal prevent the mares from becoming pregnant.

Gray and Ridgeway (10) fed 65 pregnant ewes from 0.2 to 0.8 pound of cottonseed meal for varying periods of 35 to 210 days with no ill effects except in one case where blindness and death occurred. During the four years the work was carried on 6 cases of abortion occurred among the ewes eating cottonseed meal but there were as many abortions among the ewes in the check lot which received no cottonseed meal.

The Texas Station (11) reported that 5 brood sows receiving a ration containing 15 per cent cottonseed meal seemed to stay in a good condition and produced as many pigs as did the tankage fed sows but the pigs did not grow quite as rapidly as did the tankage fed pigs.

Richardson and Green (12) found that a ration containing 50 per cent cottonseed flour, protein free milk and butterfat was sufficient for normal growth and development and reproduction to the third generation.

EXPERIMENTAL

This experiment was begun in 1926 in order to determine the effect of heavy feeding of cottonseed meal with ample roughage of

high quality on growth, health, reproduction, and milk production of dairy cattle over a period of three generations. The effects of cottonseed meal feeding on growth and health of the first generation from 3 to 18 months have been previously reported (1), and also the effect of heavy feeding of cottonseed meal on the consistency of feces of dairy cattle (13). The present paper reports the effect of heavy feeding of cottonseed meal on health, reproduction, and milk production of dairy cattle for the first generation up to approximately four years of age. The first generation of animals used in this investigation consisted of 10 high grade Holstein heifers. They were divided into 2 lots of 5 animals each.

Animals in lot I, G-1, G-3, G-5, G-7, and G-9, received cottonseed meal as the principal source of protein, whereas in lot II, G-2, G-4, G-6, G-8, and G-10, received linseed oil meal as the principal source of protein. Linseed oil meal was fed as a check against cottonseed meal since the former is recognized as a safe protein concentrate.

The inheritance of the animals in both lots was similar. Eight of the animals, G-3 to G-10 inclusive, were sired by the same bull. Animal G-1 and the dam of G-10 were also sired by the same bull. The dams of G-3, G-4, G-5, and G-6 were sired by the same bull as well as the dams of G-7, G-8, and G-9. Animals G-7 and G-8 are twins. The milk records of the dams of the animals varied from 7,343 pounds to 9,440 pounds.

Rations

The cottonseed meal used in this investigation was purchased on the open market. Up to the first calving time the animals in lot I received sufficient cottonseed meal in the ration to meet the Armsby requirements for protein. Since a slightly smaller amount of cottonseed meal was fed than linseed meal in order to get the same amount of protein, a small amount of yellow corn was fed to lot I to bring the total digestible nutrients up to the same level as contained in the larger amount of linseed oil meal. Corn silage and good quality timothy hay were fed as roughage in whatever amounts the animals would consume. The timothy hay used was high grade No. 2 as to color and No. 1 as to purity. The pro-

tein in the corn, corn silage, and timothy hay was considered as excess protein. The animals in lot II were fed similar to those in lot I except old process linseed oil meal replaced the cottonseed meal in the ration. Two per cent bone meal and one per cent salt was added to the protein concentrates fed.

After first calving the animals were fed similary except the requirements for maintenance and milk production were calculated according to the upper limits of the Morrison Feeding Standard.

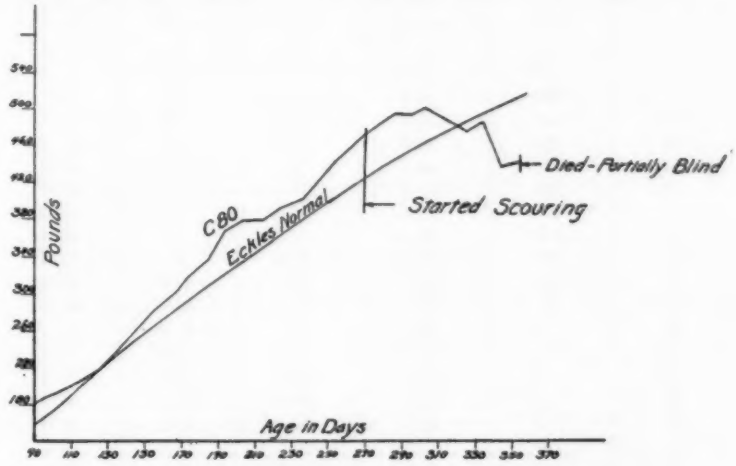

FIG. 1. SHOWING EFFECT OF THE COTTONSEED MEAL USED IN THIS INVESTIGATION ON GROWTH AND HEALTH OF C-80 WHEN FED WITH ROUGHAGE OF POOR QUALITY (WHEAT STRAW)

In cases where the cottonseed meal or linseed meal, silage, and timothy hay consumed did not furnish sufficient total digestible nutrients, the deficiency was made up with ground yellow corn.

Production of cottonseed meal injury

The cottonseed meal used in this investigation was the same as that fed to calf C-80. This Holstein bull calf was placed on a ration of cottonseed meal, wheat straw, corn, oats, bone meal, and salt at 94 days of age. Skim milk was also fed until 150 days of

age. Figure 1 shows the growth in weight of this animal compared to Eckles' normal (14).

Food consumption is shown in table 1. This animal died at 361 days of age with the characteristic symptoms of cottonseed meal

TABLE 1

Showing feed record of calf C-80, which was fed cottonseed meal from the same shipment as was used in this investigation

10-DAY PERIODS	SKIMMILK	COTTONSEED MEAL	CORN	OATS	WHEAT STRAW
	pounds	*pounds*	*pounds*	*pounds*	*pounds*
1	120	3.7	9.5	9.5	12.0
2	120	9.0	5.0	5.0	10.0
3	120	14.6	5.0	5.0	13.0
4	120	20.1	5.0	5.0	19.5
5	120	23.8	5.0	5.0	20.0
6	120	26.6	5.5	5.5	20.0
7	120	27.0	7.0	7.0	20.0
8	120	28.8	7.5	7.5	20.0
9	120	30.0	8.3	8.3	20.0
10	120	31.2	8.5	8.5	23.0
11	120	32.0	10.0	10.0	30.0
12	26	34.7	10.0	10.0	30.0
13	0	39.7	10.0	10.0	30.0
14	0	40.0	10.0	10.0	30.0
15	0	42.0	10.0	10.0	30.0
16	0	45.0	10.0	10.0	30.0
17	0	47.5	10.0	10.0	30.0
18	0	50.0	10.0	10.0	30.0
19	0	51.0	10.0	10.0	30.0
20	0	52.6	10.0	10.0	30.0
21	0	54.0	10.0	10.0	25.0
22	0	55.0	7.5	7.5	10.0
23	0	35.5	5.0	5.0	8.0
24	0	44.0	7.0	7.0	10.0
25	0	52.5	7.5	7.5	10.0

injury. It was emaciated, scoured badly, and became partially blind about six weeks before death. An examination of table 1 will also reveal that the calf had a poor appetite during the last thirty days of life.

Cottonseed meal consumption

Since cottonseed meal was fed in sufficient amounts to meet the entire protein requirements for maintenance and lactation, quite

large amounts of the meal were fed daily, especially during heavy milk production. As much as 11 pounds of cottonseed meal were fed per day during the first months of lactation. Food consumption for both lots is shown in table 2. G-3 consumed an average of 7.3 pounds of cottonseed meal per day from the first to second calving time. On the same basis G-1 consumed 7.1 pounds of cottonseed meal; G-5 6.8 pounds; G-7 6 pounds; and G-9 5.9 pounds. Such heavy consumption of cottonseed meal is far beyond the amounts recommended or fed to dairy cattle over a

TABLE 2

Showing feed record of the two lots of animals used in this investigation

	6 MONTHS TO FIRST CALVING							FIRST TO SECOND CALVING						
	Days	C.S.M.	Average daily	L.O.M.	Corn	Silage	Timothy	Days	C.S.M.	Average daily	L.O.M.	Corn	Silage	Timothy
Lot I														
G-1	560	1,373	2.45	—	154	7,438	4,554	371	2,629	7.1	—	1,123	9,110	4,438
G-3	541	1,266	2.34	—	220	6,958	4,460	361	2,636	7.3	—	1,141	8,478	4,652
G-5	542	1,259	2.32	—	277	6,002	4,289	407	2,777	6.8	—	1,262	8,859	4,691
G-7	550	1,288	2.34	—	260	6,526	4,185	462	2,768	6.0	—	881	8,820	5,273
G-9	580	1,352	2.35	—	258	7,193	4,756	517	3,060	5.9	—	1,777	9,607	5,874
Lot II														
G-2	564	—		1,682	14	7,416	4,721	362	—		2,563	218	8,843	4,204
G-4	596			1,730	81	7,598	4,845	428			3,790	1,158	8,170	5,254
G-6	551			1,565	48	6,729	4,399	481			3,412	510	9,427	5,717
G-8	547			1,589	100	6,389	4,288	468			4,063	932	10,352	5,649
G-10	614			1,808	50	8,533	5,553							

long period of time. Since the two protein concentrates were fed to meet the entire protein requirments for maintenance and milk production the protein in the ground corn, silage, and timothy hay might be considered as excess protein. The animals in both lots, therefore, consumed about 50 per cent more protein than required by the upper limits of the Morrison Standard.

RESULTS

Health

There has been very little difference in the health of the 2 lots of animals. The heavy feeding of cottonseed meal has not produced

TABLE 3

Showing reproduction record

	Age of first oestrum	First calving						Second calving						Third calving		
		Number of services	Blood reaction	Days carried calf	Weight of calf at delivery	Health of calf at delivery	First oestrum period after calving	Number services	Blood reaction	Days carried calf	Weight of calf at delivery	Health of calf at delivery	First oestrum period after calving	Number services	Blood reaction	
Lot I. C.S.M.:																
G-1	357	2	Neg.	275	90	Normal	59	1	Neg.	271	87	Normal*	48	2	Neg.	Pregnant
G-3	430	1	Neg.	272	87	Normal	28	1	Neg.	275	82	Normal	75	5	Neg.	Pregnant
G-5	385	1	Neg.	277	87	Normal	17	2	Neg.	278	95	Normal	128	3	Neg.	Pregnant
G-7	439	1	Neg.	270	63	Normal	84	1	Neg.	272	72	Normal	132	1	Neg.	Pregnant
G-9	425	1	Neg.	275	75	Normal*	124	3	Neg.		84	Normal			Neg.	Open
Average	403			274	80.4		62									
Total		6						8								
Lot II. L.O.M.:																
G-2	221	2	Neg.	277	92	Normal	47†	2	Neg.	276	88	Normal*	25	1	Neg.	Pregnant
G-4	407	3	Neg.	276	79	Normal	32	2	Neg.	287	81	Normal	93	1	Neg.	Pregnant
G-6	414	1	Neg.	277	79	Normal	29	3	Neg.	270	79	Normal	87	2	Neg.	Open
G-8	430	1	Neg.	276	80	Normal	79	2	Neg.	240	34	Weak, died	Cow died after previous abortion, uterus inverted			
G-10	493	2	Neg.	281	88	Normal	11	4	Pos.	Pregnant						
Average	393			277	85.6		40									
Total		9						12								

* Died 48 hours after birth, due to *B. coli* infection.

† Date of first oestrum period not noted, calculated back 28 days.

symptoms of cottonseed meal injury. G-8 in lot II, which received linseed oil meal, aborted and had an inverted uterus at second calving time. She was negative to the abortion test. It is difficult to determine whether the abortion was due to the ration since none of the other animals receiving the same ration have reacted similarly. After two weeks an infection developed which resulted in death. This infection cannot be attributed to the ration.

TABLE 4

Showing milk and fat production (305 days per lactation)

	FIRST LACTATION		SECOND LACTATION	
	Milk	Fat	Milk	Fat
	pounds	pounds	pounds	pounds
Lot I. C.S.M.:				
G-1	9,803	313.6	12,184	376.1
G-3	10,279	361.4	10,431	348.0
G-5	10,268	387.8	12,632	369.3
G-7	7,613	284.5		
G-9	10,440	380.4		
Average	9,861			
Lot II. L.O.M.:				
G-2	6,340	235.1	8,744	342.1
G-4	11,190	413.2	12,786	488.3
G-6	7,932	279.0		
G-8	9,348	331.3		
G-10	6,208	189.7		
Average	8,204			

Reproduction

The reproduction records of both lots are summarized in table 3. The birth weights of the calves from lot I compare favorably with those in lot II. G-7's calf in lot I was small, which was probably due to inheritance since the calf's dam was also the smallest animal of either lot.

The loss of 3 calves, 2 in lot I and 1 in lot II, can in no way be attributed to the effects of the ration upon the dams, since the calves were strong and active at birth. All 3 calves died at about

48 hours of age. Apparently a very virulent type of *Bacillus coli* (*Escherichia coli*) was present in the herd at the time these calves were affected. *B. coli* was cultured from the organs and glands of G-2's calf of lot II. Two other calves not on this experiment, born about the same time, died with similar symptoms.

G-8 of lot II which received linseed oil meal aborted her second fetus. The calf weighed only 35 pounds, was very weak, and died in forty-eight hours. The dam was negative to the Bacillus abortus test.

G-1 of Lot I retained her placenta at second calving but passed it normally at third calving.

There was no significant difference between the two lots from the standpoint of reproduction.

Milk production

Milk and butterfat records are shown in table 4. Three animals in lot I receiving cottonseed meal produced more than 10,000 pounds of milk during the first lactation while only one animal in lot II receiving linseed oil meal produced that amount. The animals in lot I which received cottonseed meal produced more milk and butterfat. These differences are probably due to individual variation.

Mastitis

It is a common impression among dairymen that cottonseed meal when fed heavily produces mastitis. In order to determine whether the heavy feeding of cottonseed meal caused an increased number of bacteria in the udder, the bacteria in the milk from each of the animals in both lots was determined over a period of 6 months. Samples were taken for 3 consecutive days each month at the evening milking. The milk of each animal was drawn into a sterile tube in approximately equal amounts from each quarter. One cubic centimeter of milk was then pipetted in 9 cc. of sterile physiological salt solution. One cubic centimeter of this dilution was then plated in duplicate, using nutrient agar. Plates were incubated for 48 hours at room temperature and then incubated for forty-eight hours at 37°C. Results are shown in table 5.

TABLE 5

Showing the number of bacteria per cubic centimeter of freshly drawn milk

DATE	LOT I. COTTONSEED OIL										LOT II. LINSEED OIL MEAL									
	G-1		G-3		G-5		G-7		G-9		G-2		G-4		G-6		G-8		G-10	
	Average daily C.S.M. fed	Bacteria per cubic centimeter	Average daily C.S.M. fed	Bacteria per cubic centimeter	Average daily C.S.M. fed	Bacteria per cubic centimeter	Average daily C.S.M. fed	Bacteria per cubic centimeter	Average daily C.S.M. fed	Bacteria per cubic centimeter	Average daily L.O.M. fed	Bacteria per cubic centimeter	Average daily L.O.M. fed	Bacteria per cubic centimeter	Average daily L.O.M. fed	Bacteria per cubic centimeter	Average daily L.O.M. fed	Bacteria per cubic centimeter	Average daily L.O.M. fed	Bacteria per cubic centimeter
1928																				
October 17	4.5	Dry	6.4	400	7.2	80	6.9	30	3.0	15	3.0	Dry	9.4	295	8.4	25	9.9	25	3.0	Not calved
October 18		Dry		1,660		65		40		100		Dry		885		65		60		Not calved
October 19		Dry		3,320		260		30		60		Dry		980		65		70		Not calved
November 14	2.2	Dry	2.0	Dry	7.0	65	6.0	50	6.0	25	2.8	Dry	8.8	265	7.2	75	9.4	40	3.0	Not calved
November 15		Dry		Dry		430		15		10		Dry		200		30		15		Not calved
November 16		Dry		Dry		155		5		120		Dry		190		10		45		Not calved
December 18	7.5	15	2.0	Dry	5.8	130	6.4	10	9.9	140	10.5	315	8.0	305	7.0	15	9.4	75	4.5	65
December 19		10		Dry		160		40		225		1,610		130		65		235		140
December 20		15		Dry		125		10		185		525		50		20		35		65
1929																				
January 18	10.8	20	11.7	970	2.0	Dry	6.2	170	10.2	285	10.5	205	9.6	20	6.8	20	8.4	40	11.4	60
January 19		40		120		Dry		20		195		270		55		130		70		110
February 25	10.2	30	11.8	960	2.0	Dry	5.6	15	9.9	25	10.6	5	2.6	Dry	6.8	5	8.8	15	9.0	15
February 26		30		4,920		Dry		10		60		0		Dry		15		5		10
February 27		20		2,890		Dry		5		20		5		Dry		15		10		10
March 19	9.9	60	10.5	2,870	6.0	25	5.3	45	6.6	70	10.5	200	2.6	Dry	6.4	85	8.2	30	9.3	25
March 20		40		2,250		25		40		35		125		Dry		20		0		10
March 21		20		400		15		165		20		60		Dry		15		5		5

Apparently all bacterial counts were within a normal range with the exception of one animal, G-3 in lot I, which had a rather high count. An examination of the milk from the separate quarters revealed a high count in one quarter. However, this cow has never shown any evidence of mastitis. The animals in both lots have been free from this disease although other animals in the experimental herd have had mastitis. Milking machines were used in milking the animals.

DISCUSSION OF RESULTS

The results of this investigation lend further support to the theory set forth in a previous paper (1) that cottonseed meal injury in dairy cattle is due to a dietary deficiency caused by lack of a factor or factors carried by good quality hay. In the previous paper (1) symptoms of cottonseed meal injury were produced in two calves which did not receive cottonseed meal in the ration. The North Carolina Station (8) reported death of animals fed linseed oil meal, peanut meal, and soybean meal, as well as with cottonseed meal where a poor quality of roughage was used. The cottonseed meal fed in this investigation produced symptoms of cottonseed meal injury in calf C-80 where a poor quality of roughage was fed. However, when a good quality of roughage was fed, large amounts of cottonseed meal were consumed by the animals in this experiment without injury.

The results fail to support a commonly accepted idea that the heavy feeding of cottonseed meal to dairy cattle results in so called injury due to the presence of a poisonous principle, gossypol. The cottonseed meal used in this investigation was purchased on the open market. As much as 11 pounds of cottonseed meal were fed per day during the first few months of lactation without injury. G-1 consumed 7.1 pounds; G-3 7.3 pounds; G-5 6.8 pounds; and G-7 6 pounds of cottonseed meal on the average per day from first to second calving, which includes a two months dry period. The health of these animals have been good thus far.

Possibly gossypol is either non-toxic to the bovine or in the process of extraction of the oil from the raw cottonseed sufficient of the "free" gossypol is converted to the "bound" gossypol to

render the meal sufficiently non-toxic for use by dairy cattle. This problem is under investigation at the present time at this Station. In connection with toxicity of cottonseed meal for different species Gray and Ridgeway (10) noted that the cottonseed meal which they fed ewes with no ill effects caused the death of several hogs in the swine experimental work.

Milk production has not been interferred with by heavy consumption of cottonseed meal. G-1 produced 9,803 pounds; G-3 10,279 pounds; G-5 10,268 pounds; G-7 7,613 pounds; and G-9 10,440 pounds of milk during the first 305 days of the first lactation period.

The heavy feeding of cottonseed meal to dairy heifers from 90 days to approximately four years of age resulted in normal reproduction when compared with the check group which received linseed oil meal along with timothy hay, corn silage, and yellow corn.

There was no significant difference between the 2 lots in strength of calves at birth. Two calves from lot I fed cottonseed meal and one calf from lot II fed linseed oil meal died at 48 hours of age, although they appeared normal and healthy at birth. The cause of death was thought to be due to the presence of a virulent *B. coli* infection in the herd, since 2 other calves born about the same time and not on this experiment died with similar symptoms. *B. coli* was cultivated from the organs of G-2's calf whose dam received linseed oil meal. Several calves not on this experiment have died with similar symptoms since then. *B. coli* was cultivated also from these calves.

G-8 which received linseed meal aborted her second fetus with no apparent cause. This is the only calf in either group which was not strong at birth. There was but little difference between the birth weights of the calves of the two lots.

Although the animals used in this investigation were kept among animals positive to *B. abortus* test, all of the animals in both lots have remained free from the disease except G-10 receiving linseed oil meal, which became positive but has not aborted. The heavy feeding of cottonseed meal has not made these animals more susceptible to *B. abortus* infection. The results indicate that the liberal feeding of cottonseed meal does not affect repro-

duction adversely when fed with ample hay of high quality and corn silage.

The heavy feeding of cottonseed meal did not increase the susceptibility of heavy milking cows to udder infection. In previous work the normal number of bacteria in the udder of cattle was determined by Copeland and Olson (15) who found that milk drawn from each quarter of the udder of 40 cows gave an average count of 1,541 per cc. of milk. Extreme bacterial counts ranged from 0 to 347,000. Harding and Wilson (16) examined 1,230 samples of milk from the udders of 78 normal cows. They found an average of 428 bacteria per cc. of milk.

In this investigation all the bacterial counts were within normal range except for G-3 in lot I which received cottonseed meal. However, no evidence of mastitis was observed in this animal. These results were secured even though the animals used in this investigation were kept with other cows which had mastitis. Our results are not in accord with those secured by Moore (2) who reported that the heavy feeding of cottonseed meal caused mastitis and loss of quarters, whereas little trouble occurred in a lot which did not receive cottonseed meal although all lots produced similarly. Moore did not state whether or not both lots were equally exposed to infection.

SUMMARY

The results of this investigation lend further support to the theory that cottonseed meal injury in dairy cattle is due to the lack of a factor or factors carried by good quality hay.

Liberal feeding of cottonseed meal to dairy cattle from three months to four years of age along with ample hay of high quality and corn silage had no apparent effect on health, reproduction or lactation.

Liberal feeding of cottonseed meal to lactating cows did not increase susceptibility to udder infection.

REFERENCES

(1) Reed, O. E., Huffman, C. F., and Addington, L. H.: Cottonseed meal as a feed for dairy calves. Jour. Dairy Sci., 1928, xi, 488–515.

(2) Moore, J. S.: Feeding cottonseed meal and hulls to dairy cows. Miss. Agr. Exp. Sta. Bul. 174, 1914, 1–10.
(3) Coombs, S., and Curtis, R. S.: The effect of cottonseed meal upon the growth and reproduction of cows. Jour. Dairy Sci., 1921, iv, 334–342.
(4) Forty-sixth Annual Report, N. C. Agr. Exp. Sta, 1923, 63.
(5) Forty-seventh Annual Report, N. C. Agr. Exp. Sta., 1924, 55–56.
(6) Forty-eighth Annual Report, N. C. Agr. Exp. Sta., 1925, 23.
(7) Forty-ninth Annual Report, N. C. Agr. Exp. Sta., 1926, 32–35.
(8) Fiftieth Annual Report, N. C. Agr. Exp. Sta., 1927, 42–48.
(9) Bell, G. A., and Williams, J. O.: Cottonseed meal for horses. U. S. D. A Bul. 929, 1920.
(10) Gray, D. T., and Ridgeway, J. W.: Feeding cottonseed meal to pregnant ewes. Ala. Polytechnical Institute Exp. Sta. Bul. 148, 1909.
(11) Cottonseed meal for maintaining, growing and fattening hogs. Fortieth Annual Report, Texas Agr. Exp. Sta., 1927, 71.
(12) Richardson, A. E., and Green, H. S.: Nutrition investigations upon cotton seed meal. II. Jour. Biol. Chem., 1916, xxx, 243–258.
(13) Huffman, C. F., and Moore, L. A.: Effect of cottonseed meal and linseed oil meal on the consistency of feces of dairy cattle. Jour. Dairy Sci., 1929, xii, 410–418.
(14) Eckles, C. H.: The normal growth of dairy cattle. Missouri Agr. Exp. Sta. Res. Bul. 36, 1920.
(15) Copeland, L., and Olson, T. M.: The bacterial flora of normal cows' udder. S. Dak. Agr. Exp. Sta. Bul. 218, 1926.
(16) Harding, H. A., and Wilson, J. K.: A study of the udder flora of cows. New York Agr. Exp. Sta. Geneva Tech. Bul. 27, 1913.

PLATE 1

SHOWING G-3 OF LOT I AT 4 YEARS OF AGE JUST PREVIOUS TO 3RD CALVING

This animal consumed an average of 7.3 pounds of cottonseed meal daily from first to second calving.

PLATE 2

SHOWING STRENGTH OF G-9'S CALF OF LOT I AT BIRTH

This calf died at forty-eight hours of age due to B. coli infection.

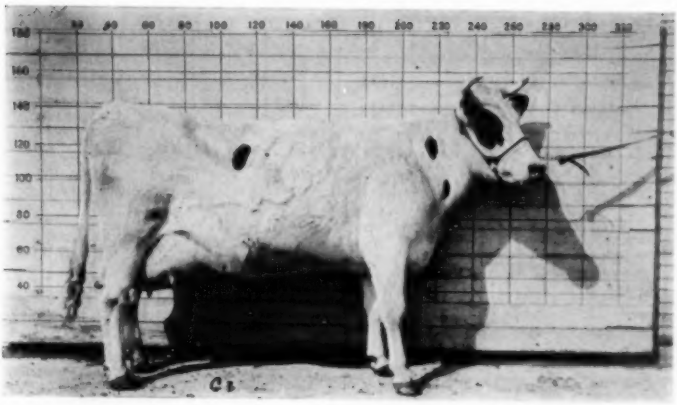

PLATE 1

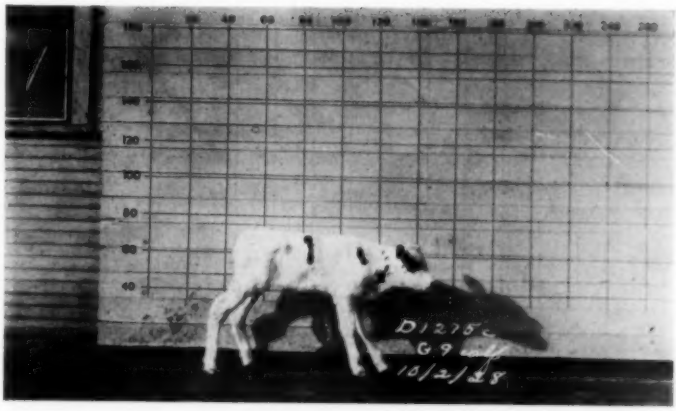

PLATE 2

FAT SOLUBLE VITAMINS

XXXI. BUTTER FAT: ITS ANTIRACHITIC PROPERTIES AND ITS ARTIFICIAL ACTIVATION*

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WITH THE COÖPERATION OF BLANCHE M. RIISING

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For many years the inference might have been drawn from general premises that butter fat is low in antirachitic potency. If butter fat were not poor in this dietary essential the incidence of rickets in children fed on cows' milk should be a very rare occurrence, but as a matter of fact it is well known that the vast majority of infants fed cows' milk develop rickets during the winter months.

Some years ago, however, Mellanby (1) in his pioneering experiments was very much impressed with the power of butter fat in protecting puppies against deficient calcification of bone. But he found that cod liver oil was far superior. McCollum, Simmonds, and Becker, and Shipley (2) found that while butter fat contained the calcium depositing factor, it was present in much smaller amounts than in cod liver oil and in other fish oils which they examined. McCollum, Simmonds, Shipley, and Park (3) found that 3 per cent of cod liver oil prevented the occurrence of rickets while even 20 per cent of butter fat failed to induce normal bone growth. Jones, Steenbock, and Nelson (4), seeking to duplicate the experimental work of others, which was designed to point out the disproportionate activity of butter fat and cod liver oil in their ability to cure ophthalmia as contrasted with rickets, came to the conclusion that cod liver oil might in certain instances be 200 times as active as butter fat in inducing normal calcification of bone.

In 1924 experiments from this laboratory (5–10) as well as those from the laboratory of Hess (11–16) showed definitely that

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the antirachitic potency of numerous fats could be increased by exposure to ultra violet radiations such as those produced by a quartz mercury vapor lamp.

Steenbock and Black (17) showed that butter fat could be made decidedly antirachitically active by the same procedure. They also showed that oils such as cocoanut oil, corn oil, oleo oil, peanut oil and cottonseed oil, which had become rancid, could not be made potent by such exposure. This was taken as evidence that the induction of antirachitic properties in our ordinary fats was not due to the action of the ultra violet irradiations upon the fats themselves, but upon certain substances of a rather labile character contained in solution in the fats and oils. Steenbock, Black, and Nelson (8) had furthermore previously reported that apparently all the antirachitic potency found in irradiated fats was localized in the unsaponifiable constituents contained therein. In the same year (9) they obtained evidence that while sterols of ordinary purity and even after numerous recrystallizations could become antirachitically activitated, in view of the fact that sterols are separated from one another with such great difficulty, the evidence that all activation was confined to the action of the ultra violet rays upon cholesterol, for example, was not at all convincing (17). Therefore it was generally referred to the activation of unsaponifiable lipoids as a group (6). That sterols were apparently activated was also reported by Hess and Weinstock (15). Hess, Weinstock, and Helman (16) furthermore separated an active unsaponifiable constituent from irradiated linseed oil.

While it thus became generally known that antirachitic activation was effected not by the action of the ultra violet rays upon proteins, carbohydrates and fats, but upon certain unsaponifiable constituents, presumably sterols, it remained for Windaus and Hess (18), Rosenheim and Webster (19, 20, 21), and Heilbron, Kamm, and Morton (22) to discover that the activation was resident in a sterol known as ergosterol, which apparently occurs widely distributed in small amounts, being concentrated in the unsaponifiable fraction and frequently crystallized out with other plant and animal sterols. While formerly, positive antirachitic tests were obtained when a milligram of ordinary choles-

terol was fed to rachitic animals, with irradiated ergosterol it was found possible to obtain results with 0.0001 mgm. or even less as a single dose.

It is still an open question (23–26) whether other substances besides ergosterol can be made active by ultra violet irradiation. Part of the difficulty in determining this lies in the fact that chemical tests for sterols in general are infinitely less delicate than the biological tests, and furthermore, inasmuch as it has been shown that derivatives of sterols or sterol mixtures can be activated (9, 20) it must be recognized that highly purified activatable starches and proteins may owe their activatability to their existence in combination with ergosterol. But to the present time it has not been found possible in this laboratory to prepare certain non-activatable starches, for example, by long continued extraction with ether and alcohol which should extract free ergosterol.

In the experiments discussed in this paper, presentation of results and experience in the use of the irradiation process is limited to butter fat. Here the problem simplified itself materially because in the irradiation of butter fat we are not concerned with its effect upon other substances, such as enzymes, proteins or the water soluble vitamins which might be injured. Primarily the problem resolved itself into defining a treatment effective in the induction of antirachitic activity without concomitant destruction of vitamin A, palatability, or color. It was soon determined that the process of irradiation need not be carried out for such a length of time or with such intensity as to destroy palatability or to cause bleaching, because the maximum antirachitic potency can be induced in far shorter time than that which will cause the aforementioned undesirable changes. The most difficult conditions which had to be met were those which concerned themselves with the prevention of vitamin A destruction. We found it necessary to carry out a rather extended series of experiments to define the conditions accurately.

EXPERIMENTAL

Our first experiments concerned themselves with the definition of the conditions necessary to secure maximum activation of

butter fat. We reserved for our experiments fifty pounds of June butter fat manufactured in the University Creamery from the milk of cows on pasture. This was kept in cold storage and small samples were melted rapidly in an oven at approximately 95°C., filtered through paper and then directly irradiated as required. For the irradiation there was used for the most part an Alpine Sun Lamp kindly loaned us by the Hanovia Chemical and Manufacturing Company. In some of our experiments, however, we also used a Cooper-Hewitt mercury vapor lamp of the B.Y. type. The distance of exposure was kept uniform at 18 inches. The butter fat was kept melted during the exposure, usually being kept at a temperature of approximately 35°. In no case was the temperature allowed to exceed 50° because of possible harmful effects. The exposure was always carried out in pie tins $7\frac{3}{4}$ inches in diameter. The pie tins were kept bright in order that the rays penetrating the butter fat completely might reflect their action from the bottom of the container. Though not proven, it was believed that this might be a very important factor because for the most part our experiments were limited to the exposure of a layer of butter fat not less than 0.08 cm. in thickness and not more than 1.28. It was not found practical to use thinner films or layers because of the difficulty of maintaining uniform distribution of the fat on the bottom of the containers. Outside of the variations in depth of material, we also introduced a time variable. This ranged from 10 minutes to 16 hours.

The antirachitic potency of the treated butter fat was determined with the technique developed in this laboratory in the course of the last few years. All the animals, namely rats, used for the experiments were produced in the laboratory on our modified stock ration consisting of yellow corn 71, linseed oil meal 15, casein 5, alfalfa meal 2, bone ash 1, sodium chloride 1, butter fat 5, fed with fresh milk and water ad libitum. Litters produced by animals on this diet were reduced to 6 in number when they were a week old, and were used for the experiment only after they had attained a weight of approximately 60 grams in not more than 4 weeks from the time of birth.

Two types of experiments were used for the testing of the irra-

diated product. The one given first consideration was based upon the Johns Hopkins technique (27) in which the diagnosis of the curative properties of a treated product is dependent upon the deposition of calcium in the metaphyses of animals in which rickets had been produced by feeding our 2965 ration (17). In this technique the animals are fed the aforementioned ration consisting of yellow corn 76, wheat gluten 20, calcium carbonate 3, sodium chloride 1, for a period of not less than 3 weeks. Sometimes at 3 weeks, though more often at about 24 to 25 days, young rats have developed a severe rachitic condition indicated not only by enlargement of joints such as the wrists, but also by a very shambling, so-called rachitic gait. When animals with this degree of rachitic involvement have introduced into their 2965 ration an antirachitically active agent such as cod liver oil, irradiated cereals, or fats in sufficient amounts, calcium deposition in the form of a calcium phosphate complex occurs in the rachitic lesions, this being especially discernible in the metaphyses of the long bones.

In the technique as used in our laboratory, we keep the animals for a period of 10 days after the introduction of the irradiated substance into the diet before we make a diagnosis. Usually we have made it a practice to feed all of the addition in the first 50 grams of ration fed in this period. This secures complete consumption of the antirachitic addition because animals usually consume in the neighborhood of 7 grams daily. Due precautions are taken that the observed results will not be complicated by a starvation factor, because it is well known that starvation even for a few days will likewise produce a healing of the lesions. For this purpose consumption records of the animals are kept daily. At the end of the 10-day period the animals are killed with ether, and the wrists, after dissecting away skin and muscles, are placed in 10 per cent formaldehyde for a period varying from 4 hours to as many days. They should not be kept for many weeks because this has frequently been found to result in the resolution of the calcium deposits. Short of this, at any time after 4 hours, the radii and ulnae ends are split with a sharp scalpel, then they are immersed in a 1.5 per cent solution of silver nitrate and exposed to a Mazda

light. With deposition of silver in the area of the bone tissue impregnated with lime salts, the bones are removed to water and kept there for observation. The extent of the calcium deposition in the rachitic metaphyses as revealed by this technique is used as a measure of the antirachitic potency of the material tested.

In these experiments we used butter fat which had been irradiated at 0.08, 0.16, 0.32, 0.64, and 1.28 cm. in depth for periods of 30 minutes and 2 hours. It was fed at levels of intake of 20, 40, 80 and 120 mgm. daily, separately and continuously for 10 days instead of being incorporated in the ration. We had frequently observed decided variations in the results obtained by the Johns Hopkins method (27) and it was for that reason that we projected our experiments on such an extensive scale. For the entire series we used a total of 43 litters of rats, all of which had been produced with our standard technique in the laboratory, and all of which were kept under as near identical conditions as we found it possible to define them.

The data accumulated as a result of this work are presented in part in table 1 for the reader's own deductions. They were selected as a group without elimination of any of the experiments of the series, so that they can be taken as representative of the results obtained as a whole. As each litter of rats contained only 6 individuals, it was, of course, experimentally impossible to make all comparisons of the different depths of exposure as well as time intervals within each litter. The table therefore shows in its upper part, litter comparison over 30 minutes' exposure for all depths, with 2 hours' exposure with a depth of 0.32 cm. The lower part of the table shows comparisons within litters of an exposure for 30 minutes at 0.32 cm. depth, with 2 hours' exposure at all depths.

Results in the table are expressed in terms of "plus" signs; one "plus" sign is used for a narrow line of calcium deposits in the rachitic metaphyses, two for a medium line, three for a wide line, four, approximately complete healing, and five, complete healing. The data indicate in a general way that with 2 hours' exposure, the activation secured was independent of depth of butter fat exposed. With 30 minutes' exposure a pronounced

decrease in activation occurred at the greater depths, namely, 0.64 and 1.28 cm. The disconcerting revelation in all of our curative experiments was the fact that when small amounts of butter fat produced definite healing, increased intake did not result in a commensurate increase in calcium deposition. This was apparently most evident when the potency approached that necessary to produce complete healing. When mere incidence of calcium deposits was used as a criterion, the results with successive increment of dose were apparently more consistent.

From our data we are led to caution investigators who seek to use the Hopkins method (27) for quantitative purposes. In all cases, a large number of animals should be used on each level of intake; certainly the number should not be less than 6, and preferably greater. And, irrespective of the number used, the incidence of a definite continuous calcium line at a certain level of intake should be taken as the criterion in preference to successive increases in the degree of healing.

For the main program of our investigation we made use of a prophylactic type of experiment. In this technique the antirachitic supplement is added to our 2965 ration as soon as the animals are put on the experiment, which, as already stated, is done when they attain a weight of 60 grams. When very small amount of a comparatively tasteless supplement are used, so small that they cannot have an effect upon the palatability and therefore the consumption of the ration, no attempt to equalize the consumption of the ration between the various groups is made. If this is not the case, then each group of animals is given the same amount of the supplemented ration day by day, the consumption of all being determined by the lowest amount consumed by any animals in the series. This general procedure of equalized consumption is departed from only when the animal consuming the least is obviously abnormal. Usually 4 animals are put on each level of supplement and of these 4 animals each individual is from a different litter. Furthermore, every litter used in the experiments is given a representative in the group. In this manner it is believed that individual variations in litter are equalized as much as possible. At the end of 5 weeks' feeding,

TABLE 1

Antirachitic activation of butter fat as determined by cure of rickets in rats

AMOUNT FED DAILY	BUTTER FAT IRRADIATED 30 MINUTES AT DEPTHS OF					BUTTER FAT IRRADIATED 2 HOURS AT DEPTHS OF					LITTER NUMBER
	0.08 cm.	0.16 cm.	0.32 cm.	0.64 cm.	1.28 cm.	0.08 cm.	0.16 cm.	0.32 cm.	0.64 cm.	1.28 cm.	
mgm.											
20	+	+	?	−	−			+			2
	+++	+	++++	−	+			+			15
	+++		+		−			+++			27
40	++++	+++	++	−	+			+++			3
	+++	+++	+	+	+			++++			16
	+++	+++	+++	+++	?			++++			31
	++++	++++	+++	−	+			+++			35
	++	+++	+	++	+			+++			39
80	+++	++++	+++	+	++			++			6
	++++	+++	+++	+	++			+++			20
	+++	+++	++		++			+++			32
120	+++++	++	+++	+++	+++			+++			7
	++++	++++	++++	+++	++			++++			22
20			−			++++	++++	+++++	++++		9
			+			++	++	++	++	++	24
40			++			++	+++	++++	++++	++	10
			++			+	+	+++	+++		23
			++			+++	++++	+++	+++++	+++	36

80				+			++	−	+++	++++	+++	40
				+++			+++++	++++	+++	+++++	+++	12
				++			++	++	++	++++	++++	25
120				+++			−	−	+++	++	++	13
				+++			+++	+++++	+++	++++	+++	26

? = reaction questionable; + = narrow line of calcium; ++ = medium line of calcium or medium metaphyses; +++ = wide line of calcium or narrow metaphyses; ++++ = approximately complete healing; +++++ = complete healing.

the animals are killed with ether, the femora and humeri are dissected out, carefully cleaned free from adhering muscle and connective tissue, and then extracted with alcohol without drying, from 4 to 7 days. The extraction is carried out in a Soxhlet extractor the alcohol being changed at least once a day in order to remove water extracted from the bones. After this thorough extraction, the bones are dried at 100° and then ashed in an electric muffle furnace. Comparison of the antirachitic potency is based upon the percentage of ash in the bones. For control purpose, when using this technique we also frequently removed the wrists as discussed in the previously described technique, and very frequently we also examined the costo-chondral junctions for rachitic enlargement.

This method has been previously used, but the first results obtained therewith in the present experiments are shown in table 2. This table brings out the comparative induction of antirachitic potency in butter fat when irradiated with the Alpine Sun Lamp for 10 minutes as compared with 30 minutes. The irradiation in this and all subsequent experiments, unless otherwise indicated, was carried out with the butter fat at a depth of 0.16 cm. This depth was chosen provisionally because of the fact that with it, it was easy to maintain continuity of film and furthermore, the curative experiments had shown that at this depth satisfactory activation was secured with an exposure of 30 minutes. In the experiments of table 2 the butter fat was fed at a level of 10 and 20 mgm. daily intake in separate portions given to the rats daily in addition to ration 2965 fed ad libitum. Records of the consumption of ration 2965 were not kept because it was presumed that consumption would be uniform. As stated previously, each group of animals had a representative from each one of the litters used. Therefore, in the table the values in the same vertical column represent the analysis of rats from the same litter. In these experiments, both femora were analyzed in order to determine the variations in femora taken from the same animal. It will be noticed that individual analysis of the different femora taken from the same rat vary from 0.06 to 1.34 per cent. Taking the averages of each group as a whole, it is seen that 20 mgm. of

TABLE 2

Antirachitic activation of butter fat irradiated at a depth of 0.16 cm. for ten minutes as compared with thirty minutes

RAT NUMBERS	BUTTER FAT ADDITION TO RATION 2965	PER CENT ASH					GRAND AVERAGE	WIDTH OF METAPHYSES	SIZE OF COSTO-CHONDRAL JUNCTIONS
233–236	10 mgm. daily; irradiated 10 minutes	Femurs	39.0	37.81	44.20	44.31		Narrow metaphysis to almost normal epiphyseal line	Pronounced to medium enlargement
			40.07	38.82	44.57	44.01			
		Average	39.58	38.32	44.39	44.16	41.61		
237–240	20 mgm. daily; irradiated 10 minutes	Femurs	46.08	45.30	45.00	42.84		Almost normal epiphyseal line	Normal (slight enlargement in rat 238)
			46.20	44.25	43.74	43.56			
		Average	46.14	44.78	44.37	43.20	44.62		
249–252	10 mgm. daily; irradiated 30 minutes	Femurs	40.40	45.69	39.15	45.57		Narrow metaphysis	Medium to slight enlargement
			40.02	44.82	39.21	46.41			
		Average	40.21	45.26	39.18	45.99	42.66		
253–256	20 mgm. daily; irradiated 30 minutes	Femurs	45.59	49.36	49.28	49.35		Almost normal	Normal
			45.35	48.02	48.54	48.30			
		Average	45.47	48.69	48.91	48.83	47.97		

TABLE 3

Antirachitic activation of butter fat irradiated at a depth of 0.16 cm. for thirty minutes as compared with 120 minutes

RAT NUMBERS	BUTTER FAT ADDITION TO RATION 2965	PER CENT ASH					AVERAGE	WIDTH OF METAPHYSES	SIZE OF COSTO-CHONDRAL JUNCTIONS
137–140	5 mgm. daily; irradiated 30 minutes	Femurs	38.05	32.55	36.36	37.18	36.04	Trace of calcification	Pronounced enlargement
141–144	20 mgm. daily; irradiated 30 minutes	Femurs	50.11	49.12	49.42	48.12	49.19	Normal epiphyseal line	Normal
145–148	40 mgm. daily; irradiated 30 minutes	Femurs	52.76	47.66	45.94	48.01	48.59	Normal epiphyseal line	Normal
149–152	5 mgm. daily; irradiated 2 hours	Femurs	34.75	37.61	38.78		37.05	Medium metaphysis	Pronounced enlargement
153–156	20 mgm. daily; irradiated 2 hours	Femurs	49.14	49.29	44.50	49.02	47.99	Normal epiphyseal line	Normal
157–160	40 mgm. daily; irradiated 2 hours	Femurs	50.27	46.20	47.61	46.68	47.69	Normal epiphyseal line	Normal

irradiated butter fat fed daily produces more calcification than 10 mgm., and furthermore, that a 30-minute exposure of the aforementioned sample produced from 1 to 3 per cent more calcification than the 10 minute irradiation.

In table 3 are presented data obtained in the same manner as those presented in table 2 with the exception that the levels of intake were 5, 20 and 40 mgm. daily. Ordinary butter fat was irradiated for 30 minutes as contrasted with 120 minutes, the primary object of this series being to determine whether or not 30 minutes' exposure effected complete activation. Inasmuch as the femora had been found to give fairly consistent data as far as calcification was concerned in these experiments, only one femur was analyzed. Gross inspection of the table reveals immediately that 30 minutes' exposure was as efficient in inducing complete activation as 2 hours' and that with the exposure of 30 minutes, 20 mgm. of butter fat daily was sufficient to produce complete calcification.

In table 4 are presented data comparing the Alpine Sun Lamp, manufactured by the Hanovia Chemical and Manufacturing Company, with the Cooper Hewitt lamp of the B.Y. type. Both lamps when used were practically new, the former having been used for 185 hours at low intensity, while the Cooper Hewitt had been used for 82 hours. Both burners were free from blemishes and were in excellent condition. The former was run from our A.C. lighting circuit on 110 volts, as specified by the maker; the latter was run on D.C. current with a voltage of 50 and a current density of 4 amperes. The butter fat in each case was exposed under the same conditions as to temperature, container, and original source. Litter distributions among the different groups were the same as employed in previous experiments and analyses were limited to both femora.

It is seen from table 4 that while 10 minutes' exposure with the Alpine Sun Lamp gave slightly better results than 10 minutes' exposure with the Cooper Hewitt lamp, 20 minutes' exposure with the Alpine Sun Lamp gave slightly lower results than 20 minutes' exposure with the Cooper Hewitt lamp. This shows that the differences in values observed were due to experimental error or

TABLE 4

*Comparative activatability of butter fat irradiated by the Alpine Sun Lamp and by the Cooper-Hewitt Lamp**

RAT NUMBERS	BUTTER FAT ADDITIONS TO RATION 2965		PER CENT ASH IN FEMURS						GRAND AVERAGE	WIDTH OF METAPHYSES	SIZE OF COSTO-CHONDRAL JUNCTIONS
301–306	20 mgm. irradiated 10 minutes by Alpine Sun Lamp	Femurs	37.00	41.17	41.20	44.25	46.18	39.33		Medium metaphysis	Slight enlargement
			37.35	41.67	41.25	45.41	46.17	39.72			
		Average	37.18	41.42	41.23	44.83	46.18	39.53	41.73		
307–312	20 mgm. irradiated 20 minutes by Alpine Sun Lamp	Femurs	45.46	46.22	44.95	48.83	40.72	42.94		Very narrow metaphysis	Almost normal
			45.35	46.62	45.20	48.52	40.24	42.96			
		Average	45.41	46.42	45.08	48.68	40.48	42.95	44.83		
313–318	20 mgm. irradiated 30 minutes by Alpine Sun Lamp	Femurs	44.81	41.69	47.78	47.95	45.36	46.22		Very narrow metaphysis	Almost normal
			43.87	41.82	48.18	46.89	45.56	47.54			
		Average	44.34	41.76	47.98	47.42	45.46	46.88	45.64		
319–324	20 mgm. irradiated 5 minutes by Cooper-Hewitt lamp	Femurs	33.21	37.91	41.67	38.06	39.10	37.43		Medium metaphysis with cupping	Pronounced enlargement
			33.73	38.12	42.88	37.35	38.85	37.36			
		Average	33.47	38.02	42.28	37.71	38.98	37.40	37.97		
325–330	20 mgm. irradiated 10 minutes by Cooper-Hewitt lamp	Femurs	43.37	32.77	45.65	40.00	41.98	39.93		Medium metaphysis	Medium enlargement
			43.08	32.70	46.14	39.62	41.75	39.82			
		Average	43.23	32.74	45.90	39.81	41.87	39.88	40.57		
331–335	20 mgm. irradiated 20 minutes by Cooper-Hewitt lamp	Femurs	49.31	50.23	48.71	42.99	43.46			Very narrow metaphysis	Normal
			48.19	50.52	48.67	43.17	43.86				
		Average	48.75	50.38	48.69	43.08	43.66		46.91		

* Alpine Sun Lamp had been run for 185 hours (low). Cooper-Hewitt Lamp had been run for 82½ hours.

inherent variation in the calcium content of animals, from the same litters, irrespective of the antirachitic potency of the materials fed. We therefore feel safe in concluding that the quartz mercury vapor lamps of the two types compared are essentially equally proficient in effecting activation.

We have already discussed the paucity of butter fat in vitamin D content. We were therefore interested in determining the comparative potency of untreated butter fat with butter fat which had been irradiated for 30 minutes at a depth of 0.16 cm., which represents the most favorable conditions as determined by the aforementioned experiments. The results of experiments to this end are presented in table 5. Taking the usual precautions in regard to litter distribution of animals, and so forth, untreated butter fat was fed daily in individual portions contained in small dishes, separate from ration 2965 which formed the bulk of the diet of the animals. Of the untreated butter fat there were fed 80, 160, and 400 mgm. daily; of the irradiated butter fat 20, 10, and 5 mgm. The 5- and 10-mgm. portions were measured out in ether solution on small portions of ration 2965, in order to secure complete consumption. Included in the table are percentage values for butter fat which had been calculated on a basal intake assuming that every rat consumed approximately 8 grams of the ration daily. These percentage values, however, are to be taken as an approximation only. The table shows that 20 mgm. of irradiated butter fat were equal in antirachitic potency to 400 mgm. of the untreated. This represents a multiple of 20 times the activity of ordinary June butter fat as compared with the treated production.

It has already been brought out in our introduction that the excessive treatment of fats, such as olive oil and cod liver oil, under ultra violet rays brings out a total destruction of antirachitic potency. That this relation also obtains in the case of butter fat is presented in table 6. As we were not so much concerned with the quantitativeness of the reaction as with the demonstration of its occurrence, we felt that data satisfactory for our purpose could be obtained by the use of the less laborious, though more inaccurate curative experiments as contrasted with the

TABLE 5

The comparative amounts of vitamin D in butter fat natural and irradiated for 30 minutes at a depth of 0.16 cm.

RAT NUMBERS	BUTTER FAT ADDITION TO RATION 2965		PER CENT ASH IN FEMURS				GRAND AVERAGE	WIDTH OF METAPHYSES	SIZE OF COSTO-CHONDRAL JUNCTIONS
277–280	1 per cent or 80 mgm. untreated	Femurs	32.38	32.94	27.52	28.25		Wide rachitic metaphysis	Pronounced enlargement. Extreme angulation
			31.72	32.98	27.98	30.11			
		Average	32.05	32.96	27.75	29.18	30.49		
281–284	2 per cent or 160 mgm. untreated	Femurs	39.07	42.29	33.96	31.65		Wide rachitic metaphysis to medium metaphysis	Pronounced to medium enlargement
			38.78	42.25	34.18	31.42			
		Average	38.93	42.27	34.07	31.54	36.70		
285–288	5 per cent or 400 mgm. untreated	Femurs	44.76	48.52	46.70	42.16		Narrow metaphysis	Almost normal
			44.86	48.89	46.49	43.23			
		Average	44.81	48.71	46.60	42.70	45.70		
289–292	0.25 per cent or 20 mgm. irradiated 30 minutes	Femurs	47.49	48.08	43.16	45.27		Very narrow metaphysis	Normal
			46.34	47.73	42.75	45.05			
		Average	46.92	47.91	42.96	45.16	45.73		
293–296	0.12 per cent or 10 mgm. irradiated 30 minutes	Femurs	39.71	47.75	37.51	43.72		Narrow metaphysis	Slight enlargement
			39.74	47.09	36.31	43.31			
		Average	39.73	47.42	36.91	43.52	41.89		
297–300	0.06 per cent or 5 mgm. irradiated 30 minutes	Femurs	26.67	44.26	32.60	35.15		Medium metaphysis	Medium enlargement
			26.11	44.63	32.59	35.57			
		Average	26.39	44.45	32.60	35.36	34.70		

prophylactic determinations based on ash content. Butter fat for these experiments was irradiated at a depth of 0.16 cm. for 30 minutes, 5 hours, 10 hours, and 16 hours. For the short exposures

TABLE 6

The effect of excessive irradiation on the antirachitic activity of butter fat

LITTER NUMBER	RAT NUMBER	BUTTER FAT ADDITIONS TO RATION 2965			INITIAL WEIGHT	FINAL WEIGHT	AVERAGE GRAMS RATION 2965 CONSUMED DAILY	LINE TEST
			per cent	mgm.				
49	8268	Irradiated 30 minutes at 35°C.	0.5	31.0	95	90	6.2	++
	8269	Irradiated 10 hours at 35°C.	0.5	49.0	97	112	9.8	−
	8270	Irradiated 16 hours at 50°C.	0.5	38.0	96	97	7.6	−
	8271	Irradiated 30 minutes at 35° C. then heated for 15.5 hours at 50°C.	0.5	43.0	86	93	8.6	++ to +++
50	8272	Irradiated 30 minutes at 35°C.	0.5	35.5	86	88	7.1	+++
	8273	Irradiated 5 hours at 35°C.	0.5	40.0	75	80	8.0	+++
	8274	Irradiated 10 hours at 35°C.	0.5	42.5	85	92	8.5	++
	8275	Irradiated 16 hours at 50°C.	0.5	42.0	82	88	8.4	−
51	8276	Irradiated 30 minutes at 35°C.	0.5	28.0	85	80	5.6	+++
	8277	Irradiated 5 hours at 35°C.	0.5	29.0	70	68	5.8	+++++
	8278	Irradiated 10 hours at 35°C.	0.5	43.0	86	98	8.6	++
	8279	Irradiated 16 hours at 50°C.	0.5	43.5	90	98	8.7	−
	8280	Irradiated 30 minutes at 35°C. then heated for 15.5 hours at 50°C.	0.5	25.5	83	79	5.1	++++
52	8281	Irradiated 30 minutes at 35°C.	0.5	31.5	96	97	6.3	++
	8282	Irradiated 16 hours at 50°C.	2.0	118.0	98	97	5.9	−
	8283	Irradiated 16 hours at 50°C.	2.0	158.0	98	102	7.9	−
	8284	Irradiated 30 minutes at 35°C. then heated for 15.5 hours at 50°C.	2.0	148.0	92	89	8.4	++++

it was found satisfactory to keep the butter fat melted at 35°, but in the exposure of 16 hours the temperature inadvertently went up to 50°. To determine if the higher temperature in itself was a factor in the destruction of the antirachitic potency induced, we

irradiated one sample for 30 minutes at 35° and then followed this treatment with heating at 50° for 15½ hours, to make a total of 16 hours. The butter fat was incorporated in ration 2965 at levels of 0.5 to 2 per cent and these rations were fed throughout the 10-day period. Of the total intake of butter fat calculated from the total consumption of ration, the results as shown in table 6 indicated that 10 hours' exposure caused pronounced destruction, and 16 hours caused practically complete destruction as far as the low level of butter fat intake indicated. Inasmuch as activated butter fat heated for the same period of time proved to be potent, the destruction observed was not attributable to heat itself.

With the conditions for maximum activation of butter fat established, it was of interest to us to determine if this activity was a stable property under storage conditions. To obtain data on this, irradiated butter fat was stored in quart Mason jars at room temperature and in the refrigerator in the absence of light. The experiment was begun in July and terminated the following March. The room temperature was approximately 70°. By way of control some of the untreated butter fat was irradiated immediately before feeding. At the end of the storage period the butter fat stored in the ice box was found palatable and unbleached; stored at room temperature it had a slightly rancid taste. The antirachitic potency of the various samples was tested using the curative type of experiment. Although, as already stated, this method does not reveal slight differences, it was deemed sufficiently accurate for the purpose desired. The butter fat, instead of being mixed with ration 2965, was measured out in small dishes and fed daily to the animals in addition to ration 2965. The animals were weighed at the beginning and end of the test, and consumption records were kept daily. The results, as shown in table 7, revealed marked variations among the experimental groups, but without definite relation to the treatment of the butter fat. In general, the results showed that antirachitic potency induced by irradiation is a stable property.

Since the discovery that ergosterol can be activated to a high degree of antirachitic potency, it immediately suggested itself

TABLE 7

Stability of vitamin D in butter fat to storage for seven months

LITTER NUMBER	RAT NUMBER	BUTTER FAT ADDITIONS TO RATION 2965	ADDITION DAILY	INITIAL WEIGHT	FINAL WEIGHT	AVERAGE GRAMS RATION 2965 CONSUMED	LINE TEST
			mgm.				
66	8504	Irradiated, stored at room temperature	20	95	99	6.4	++
	8505	Irradiated, stored in icebox	20	102	105	7.9	+
	8506	Non-irradiated, stored at room temperature, then irradiated	20	89	94	9.9	+
	8507	Non-irradiated, stored in icebox, then irradiated	20	91	98	7.2	?
67	8520	Irradiated, stored at room temperature	20	86	94	9.7	++
	8521	Irradiated, stored in icebox	20	75	83	8.3	++
	8522	Non-irradiated, stored at room temperature, then irradiated	20	84	97	8.8	+
	8523	Non-irradiated, stored in icebox, then irradiated	20	87	92	9.6	++
	8524	Non-irradiated, stored at room temperature, then irradiated	40	94	100	9.5	++
68	8508	Irradiated, stored at room temperature	40	85	92	8.0	+++
	8509	Irradiated, stored at room temperature	40	83	92	8.6	+++
	8510	Irradiated, stored in icebox	40	90	95	8.9	++
	8511	Irradiated, stored in icebox	40	88	98	9.0	+++
	8512	Non-irradiated, stored at room temperature, then irradiated	40	98	111	8.2	+
	8513	Non-irradiated, stored in icebox, then irradiated	40	84	92	6.3	++
69	8514	Irradiated, stored at room temperature	80	79	80	7.3	+++++
	8515	Irradiated, stored at room temperature	80	82	77	5.8	+++
	8516	Irradiated, stored in icebox	80	82	81	5.7	++++
	8517	Irradiated, stored in icebox	80	95	98	7.0	++++
	8518	Non-irradiated, stored at room temperature, then irradiated	80	109	117	10.6	++
	8519	Non-irradiated, stored in icebox, then irradiated	80	98	98	8.5	+++

that a food such as butter might be activated by dissolving therein the desired amounts of activated sterol. This would have the advantage that secondary reactions of ultra violet would be entirely eliminated, and the potency would be readily controllable. Calculations show that 1 ounce of irradiated ergosterol, depending upon its purity and treatment, would suffice for the activation of from 5 to 20 tons of butter, making it the equivalent of cod liver oil in antirachitic activity.

That this is a possible method of procedure is shown in table 8. For the experiments there was used ergosterol which was irradiated in ether solution. The ether was then dissipated at a low temperature and the residue dissolved in a small quantity of melted butter fat. This was then worked up with butter in the desired amounts.

In these experiments 10 mgm. of ergosterol were used for each pound of butter. This was obviously an excessive amount, but the feeding tests showed that the preparation was not of the potency usually attained. However, even though the maximum results were not secured, our data show that this amount made the butter twice as potent as cod liver oil and 80 times as potent as the original butter, an activity obviously excessive for practical purposes but nevertheless demonstrating possibilities.

It is to be emphasized that an excessive amount of vitamin D leads to harmful consequences, manifested by excessive calcium deposition in the soft body tissues. Fortunately, the amount necessary to produce these harmful results is of the order of 1,000 times the therapeutic dose (28). Nevertheless, the supplementation of foods with activated ergosterol needs to be carefully controlled. It suggests itself that this may be done in the case of butter by restricting the marketing of a preparation used for this purpose to dilute solutions or by limiting it to colored solutions. The ergosterol, for instance, might be dissolved in butter color. Our preliminary tests have not as yet shown that it is feasible to add the ergosterol solutions in such preparations previous to churning. Further work remains to be done. Both direct and indirect activation have their shortcomings, and it remains to be determined if vitamin D can always be satisfactorily fur-

TABLE 8

The antirachitic activation of butter by the addition of irradiated ergosterol and its comparison with cod liver oil

RAT NUMBER	ADDITION TO RATION 2965	PER CENT ASH IN FEMURS					GRAND AVERAGE	WIDTH OF METAPHYSIS	SIZE OF COSTO-CHONDRAL JUNCTION
408–411	5 mgm. daily cod liver oil	Femurs	47.27	43.50	31.05	45.31		Medium to narrow metaphysis (almost a wide rachitic metaphysis in rat 410)	Slight enlargement (pronounced enlargement in rat 410)
			46.36	43.65	30.55	45.08			
		Average	46.82	43.58	30.80	45.20	41.60*		
412–415	20 mgm. daily cod liver oil	Femurs	53.93	51.09	49.90	52.27		Normal	Normal
			53.62	51.31	50.43	51.95			
		Average	53.78	51.20	50.17	52.11	51.81		
416–419	200 mgm. daily untreated butter fat	Femurs	46.66	43.33	44.18	48.40		Medium to narrow metaphysis	Medium to very slight enlargement
			46.76	42.43	43.79	48.50			
		Average	46.71	42.88	43.99	48.45	45.51		
420–423	800 mgm. daily untreated butter fat	Femurs	54.23	51.58	53.17	53.25		Normal	Normal
			54.23	52.02	53.53	53.28			
		Average	54.23	51.80	53.35	53.27	53.16		
424–427	2.5 mgm. daily butter fat mixed with irradiated ergosterol	Femurs	45.34	41.78	48.75	49.82		Narrow metaphysis (medium metaphysis in rat 425)	Slight enlargement
			45.93	42.01	48.27	51.07			
		Average	45.64	41.90	48.51	50.45	46.62		
428–431	20 mgm. daily butter fat mixed with irradiated ergosterol	Femurs	51.09	50.73	†	51.78		Normal	Normal
			51.77	50.41		51.69			
		Average	51.43	50.57		51.74	51.25		

* Excluding rat 410 average is 45.20.

† Missing.

nished in the human diet by direct irradiation. By their natural limitations of activatability, due to paucity in content of ergosterol or to lack of surface, direct irradiation of many food materials would prevent all danger of hypervitaminosis. Essentially, the advisability of the fortification of foods with vitamin D is primarily a problem of: first, what is the extent of the deficiency of the human diet in vitamin D; and second, to what extent is it absolutely essential that this deficiency be corrected? The practical fortification of the human diet in vitamin D offers no essential difficulties.

CONCLUSIONS

Curative and prophylactic techniques for the study of the antirachitic potency of butter fat demonstrated the limitations of the line test or so-called Johns Hopkins method. For quantitative work the prophylactic method, which makes use of the percentage of bone ash, does not leave open the opportunities for error through mistaken judgment. Its values are numerical and quantitative.

June butter fat was found so low in vitamin D that 5 per cent of the weight of a rickets-producing ration or an intake of about 400 mgm. per rat daily did not allow normal bone production.

June butter fat, however, did contain sufficient activatable constituents so that treatment with ultra violet radiations under suitable conditions made an intake of 20 mgm. of it per rat daily equivalent in antirachitic potency to 400 mgm. of the untreated.

A sample of cod liver oil was found about 40 times as potent as the June butter fat used in our experiments.

The activating strength of two types of quartz mercury vapor lamps, namely, the Cooper Hewitt B.Y. type and the Hanovia Alpine Sun Lamp, was found to be approximately equal.

Using a film of fat 160 mm. thick, most of the activation by exposure to the ultra violet radiations of our apparatus occurred in the first 10 minutes of exposure or less. The second 10 minutes caused some additional activation, but the effect of the third 10 minutes was questionable. After that, irradiation for a total of 2 hours or more caused no increase. When the radiation was con-

tinued for a total of 16 hours, the activation originally induced or originally found in the butter fat was totally destroyed. This indicates that at least during the latter part of the activating process activation and destruction occur simultaneously. In practice, the intensity of the ultra violet treatment can, of course, be so adjusted that the exposure need be continued for only a fraction of a second, as is now done with cereals (30), because, in any event, maximum activation is neither necessary nor, probably, desirable.

The potency of irradiated butter fat was found stable to storage, and no deleterious effects of judicious irradiation were detected.

The practicability of activating butter by the introduction of irradiated ergosterol was demonstrated.

REFERENCES

(1) Mellanby, E. 1921 Experimental Rickets, Medical Research Council Special Report, Series 61.

(2) McCollum, E. V., Simmonds, Nina and Becker, J. Ernestine. 1922 Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. Jour. Biol. Chem., liii, 293.

(3) McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A. 1921 Studies on experimental rickets. IV. Cod liver oil as contrasted with butter fat in the protection against the effects of insufficient calcium in the diet. Proc. Soc. Exper. Biol. and Med., xviii, 275.

(4) Jones, J. H., Steenbock, H., and Nelson, M. T. 1924 Fat soluble vitamins. XXII. The comparative amounts of vitamin A and antirachitic factor in butter fat and cod liver oil. Jour. Metabolic Research, vi, 169.

(5) Steenbock, H., Black, A. 1924 Fat soluble vitamins. XVII. The induction of growth-promoting and calcifying properties in a ration by exposure to ultra violet light. Jour. Biol. Chem., lxi, 405.

(6) Steenbock, H. Antirachitic Product and Process, U. S. Patent 1,680,818 filed June 30, 1924.

(7) Steenbock, H. 1924 The induction of growth-promoting and calcifying properties in a ration by exposure to light. Science, lx, 224.

(8) Steenbock, H., Black, A., and Nelson, M. T. 1924 The antirachitic activation of crude fats and other substances by exposure to light. Proc. Amer. Chem. Soc., published in Science, lx, 530.

(9) Steenbock, H., Black, A., Nelson, E. M., Nelson, M. T., and Hoppert, C. A. 1925 Antirachitic activation by light. Proc. Amer. Soc. Biol. Chemists, 1924, in Jour. Biol. Chem., lxiii, p. xxv.

(10) STEENBOCK, H., AND DANIELS, AMY. 1925 Irradiated foods and irradiated organic compounds: Therapeutic possibilities. Jour. Amer. Med. Assoc., lxxxiv, 1093.

(11) HESS, A. F. 1925 On the induction of antirachitic properties in rations by exposure to light. Science, lx, 269.

(12) HESS, A. F., AND WEINSTOCK, MILDRED 1925 Antirachitic properties imparted to inert fluids by ultra violet irradiation. Jour. Amer. Med. Assoc., lxxxiii, 1845.

(13) HESS, A. F. 1924 Experiments on the action of light in relation to rickets. Trans. Amer. Pediatrical Soc. Pittsfield, Mass., in Amer. Jour. Diseases Children, xxviii, 517.

(14) HESS, A. F., AND WEINSTOCK, M. 1924 Antirachitic properties imparted to inert fluids and to green vegetables by ultra-violet irradiation. Jour. Biol. Chem., lxii, 301.

(15) HESS, A. F., AND WEINSTOCK, M. 1925 A further report on imparting antirachitic properties to inactive substances by ultra-violet irradiation. Proc. Amer. Soc. Biol. Chemists in Jour. Biol. Chem., lxiii, p. xxv.

(16) HESS, A. F., WEINSTOCK, M., AND HELMAN, D. 1924 Oil activated by irradiation. II. Separation into an antirachitic and an inactive fraction. Proc. Soc. Exper. Biol. and Medicine, xxii, 76.

(17) STEENBOCK, H., AND BLACK, A. 1925 Fat soluble vitamins. XXIII. The induction of growth-promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. Jour. Biol. Chem., lxiv, 263.

(18) WINDAUS, A., AND HESS, A. F. 1926 Sterine and antirachitisches Vitamin. Nachr. Ges. Wiss. Göttengen Math. Physik Klasse, 175–184.

(19) ROSENHEIM, O., AND WEBSTER, T. A. 1927 XIX. The relation of cholesterol to vitamin D. Biochem. Jour., xxi, 127.

(20) ROSENHEIM, O., AND WEBSTER, T. A. 1927 The photochemical production of vitamin D from ergosterol. Lancet, cv, 2, 622.

(21) ROSENHEIM, O., AND WEBSTER, T. A. 1927. The sources of supply of vitamins A and D. Nature, September 24, 440.

(22) HEILBRON, I. M., DAMM, E. D., AND MORTON, R. A. 1927 XII. The absorption spectrum of cholesterol and its biological significance with reference to vitamin D. Part I. Preliminary observations. Biochem. Jour., xxi, 78.

(23) BILLS, C. E., AND HONEYWELL, EDNA M., and MACNAIR, W. A. 1928 Antirachitic substances. VII. Biochemical and spectroscopic studies on purified cholesterol. Jour. Biol. Chem., lxxvi, 251.

(24) KOCH, F. C., KOCH, E. M., AND RAGINS, I. K. 1929 Fractionation studies on provitamin D. Jour. Biol. Chem., lxxxv, 141.

(25) KOCH, E. M., KOCH, F. C., AND LEMON, H. B. 1929 Absorption spectra studies on cholesterol and ergosterol. Jour. Biol. Chem., lxxxv, 159.

(26) KON, S. K., DANIELS, F., AND STEENBOCK, H. 1928 The quantitative study of the photochemical activation of sterols in the cure of rickets. II. Jour. Amer. Chem. Soc., l, 2573.

(27) McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A. 1921 Studies on experimental rickets. VIII. The production of rickets by diets low in phosphorus and fat-soluble A. Jour. Biol. Chem., xlvii, 507.

(28) Steenbock, H., and Black, A. 1925 Fat-soluble vitamins. XXIII. The induction of growth promoting and calcifying properties in fats and their unsaponifiable constituents by exposure to light. Jour. Biol. Chem., lxiv, 263.

(29) Bills, C. E., and Wirick, A. M. 1930 Long time feeding experiments with activated ergosterol. I. Jour. Biol. Chem., lxxxvi, 117.

(30) Steenbock, H., Riising, B., Black, A., and Thomas, B. H 1929 Cereals and Rickets. II. Antirachitic activation of cereals. Jour. Amer. Med. Assoc., xciii, 1868.

SEASONAL VARIATIONS IN CERTAIN INORGANIC CONSTITUENTS OF DRY MILK PRODUCED IN NEW YORK AND WISCONSIN*

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The extension of nutritional and biochemical research during recent decades has brought into prominence the inherent quantitative variations of certain minor constituents of milk, both organic and inorganic. Although but meager data are as yet available directly connecting these inherent variations with the nutritive and dietetic properties of milk produced in different localities at different seasons of the year, the trend of many investigations is, nevertheless, toward a more careful study of such relationships. In the preparation of various milks designed for infant feeding, normal fluctuations in the constitution of milk which may to any degree affect the nutritive properties or physicochemical relationships during processing, are matters of concern.

Various inter-related studies carried on at this Laboratory have necessitated a determination of the limits of variation of certain inorganic constituents of the milk supply used for the preparation of a well known brand of dry milk[2] especially manufactured for infant and convalescent feeding. Inquiries from pediatrists, research institutions and individual investigators manifesting an interest in data of this character and its possible relationship to particular problems has prompted the compilation and publication of the data recorded herein.

Samples of dry milk made in representative milk producing areas of New York and Wisconsin were analyzed at monthly intervals throughout a period of one year. The test samples were

* Received for publication June 9, 1930.

[1] Collaborators assisting in obtaining analytical data were J. W. Nelson and Rita Morales, formerly employed at this Laboratory, and Lillas Myrick and Prof. J. F. McClendon of the Medical School of the University of Minnesota.

[2] Dryco Brand.

TABLE 1

Monthly variations in the ash content of dry milk produced in New York State territory

(Results expressed as percentages on moisture-free powder basis and on the ash basis)

MONTH	ASH	P_2O_5		Cl		CaO		MgO		K_2O		Na_2O		SO_3		UNDETERMINED (ASH BASIS)	O-Cl EQUIVALENT (ASH BASIS)
		Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash		
January	7.625	2.103	27.58	1.031	13.52	1.718	22.53	0.186	2.44	2.000	26.22	0.516	6.76	0.170	2.23	1.77	3.09
February	7.639	2.103	27.52	1.073	14.04	1.720	22.51	0.183	2.39	1.980	25.91	0.524	6.86	0.120	1.56	2.37	3.16
March	7.608	2.100	27.60	1.049	13.79	1.723	22.64	0.188	2.45	1.925	25.30	0.492	6.45	0.181	2.37	2.51	3.11
April	7.531	2.110	28.01	1.025	13.61	1.720	22.82	0.188	2.49	1.900	25.22	0.474	6.29	0.184	2.44	2.19	3.07
May	7.594	1.972	25.96	1.015	13.36	1.713	22.55	0.186	2.44	2.010	26.45	0.581	7.65	0.172	2.26	2.64	3.01
June	7.634	1.903	24.89	1.038	13.58	1.688	22.08	0.187	2.44	2.150	28.11	0.591	7.73	0.170	2.22	2.01	3.06
July	7.709	1.914	24.82	1.023	13.27	1.690	21.90	0.184	2.37	2.160	28.01	0.588	7.64	0.180	2.33	2.66	3.00
August	7.785	1.949	25.03	1.027	13.17	1.702	21.86	0.192	2.46	2.210	28.38	0.574	7.37	0.181	2.32	2.38	2.97
September	7.801	2.050	26.28	1.007	12.90	1.705	21.85	0.198	2.53	2.130	27.30	0.569	7.29	0.182	2.32	2.44	2.91
October	7.810	2.052	26.27	1.091	13.95	1.707	21.85	0.188	2.39	2.080	26.63	0.552	7.06	0.178	2.27	2.72	3.14
November	7.765	2.061	26.54	1.095	14.10	1.705	21.95	0.183	2.36	2.010	25.89	0.552	7.11	0.179	2.31	2.91	3.17
December	7.630	2.105	27.59	1.022	13.39	1.720	22.54	0.183	2.39	2.010	26.34	0.560	7.34	0.170	2.23	1.22	3.02
Avenue	7.678	2.035	26.50	1.041	13.55	1.708	22.24	0.187	2.44	2.047	26.70	0.548	7.14	0.172	2.24	2.24	3.05

TABLE 2

Monthly variations in the ash content of dry milk produced in Wisconsin territory

(Results expressed as percentages on the moisture-free powder basis and on the ash basis)

MONTH	ASH	P_2O_5		Cl		CaO		MgO		K_2O		Na_2O		SO_3		UNDETERMINED (ASH BASIS)	O-Cl EQUIVALENT (ASH BASIS)
		Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash	Powder	Ash		
January	7.571	2.166	28.60	0.935	12.35	1.640	21.66	0.193	2.54	2.090	27.46	0.477	6.30	0.140	1.85	2.02	2.78
February	7.606	2.152	28.29	0.934	12.27	1.665	21.89	0.196	2.58	2.100	27.60	0.457	6.01	0.180	2.37	1.75	2.76
March	7.560	2.120	28.04	0.957	12.65	1.688	22.32	0.192	2.54	2.070	27.38	0.437	5.78	0.178	2.35	1.79	2.85
April	7.534	2.104	27.93	0.928	12.32	1.702	22.59	0.189	2.50	2.060	27.24	0.459	6.09	0.176	2.34	1.67	2.78
May	7.587	2.050	27.02	0.940	12.39	1.706	22.48	0.194	2.56	2.110	27.81	0.469	6.18	0.178	2.35	2.00	2.79
June	7.629	1.987	26.05	0.879	11.52	1.715	22.48	0.190	2.49	2.270	29.75	0.498	6.53	0.170	2.23	1.54	2.59
July	7.673	1.949	25.40	0.913	11.89	1.758	22.91	0.187	2.44	2.260	29.45	0.510	6.65	0.156	2.03	1.91	2.68
August	7.761	1.979	25.49	0.989	12.74	1.762	22.70	0.203	2.62	2.210	28.47	0.499	6.43	0.187	2.41	2.01	2.87
September	7.801	2.110	27.05	0.994	12.74	1.709	21.91	0.199	2.55	2.190	28.07	0.492	6.31	0.179	2.29	1.95	2.87
October	7.737	2.121	27.41	1.041	13.45	1.724	22.28	0.197	2.55	2.100	27.14	0.470	6.07	0.175	2.26	1.87	3.03
November	7.702	2.132	27.68	1.048	13.61	1.736	22.53	0.186	2.41	2.110	27.39	0.419	5.44	0.181	2.35	1.66	3.07
December	7.610	2.140	28.12	0.990	13.01	1.679	22.06	0.192	2.52	2.100	27.59	0.439	5.77	0.162	2.13	1.73	2.93
Average	7.647	2.084	27.25	0.962	12.58	1.707	22.32	0.193	2.52	2.139	27.97	0.469	6.13	0.172	2.25	1.80	2.82

composite samples representative of the milk received at commercial milk drying establishments located in these territories, and were selected on the same day of each month throughout the year. Tables 1 and 2 and charts 1 and 2 show the variations in total ash and certain of the mineral elements. Results are

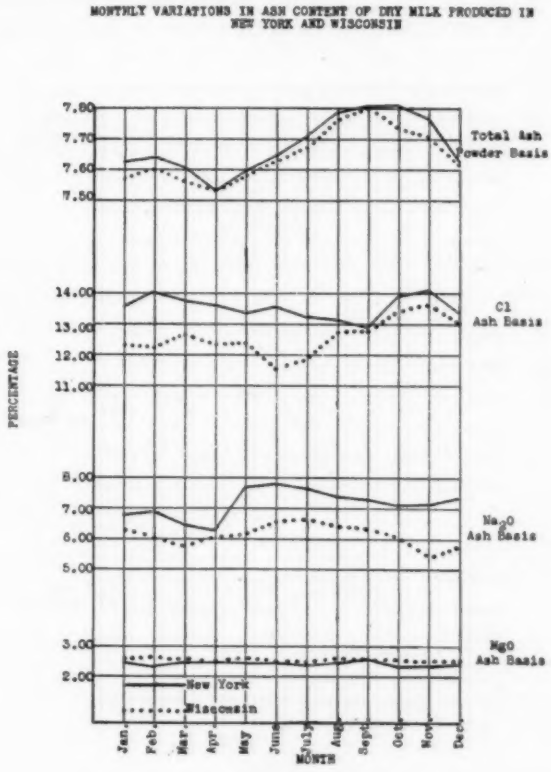

FIG. 1

expressed on the moisture-free powder basis and on the ash basis. The powder samples used for these analyses contained 12 per cent fat on the dry basis; the percentage of the various constituents may, therefore, be readily calculated for dry whole or skimmed milk, or for the reconstituted fluid products.

Although numerous analyses and special examinations for the minute traces of metals known to be normally present have been made on the milk produced in the New York and Wisconsin territories, the data as yet are too incomplete, with the possible

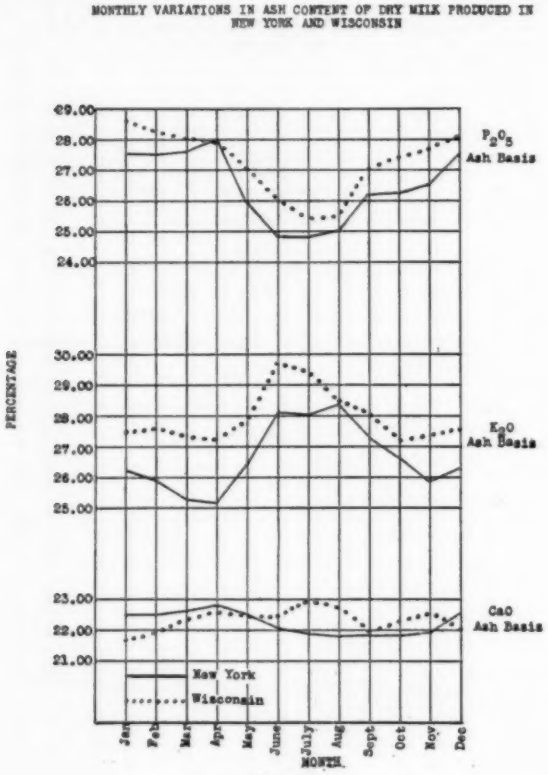

FIG. 2

exception of those pertaining to the iodine content, to warrant conclusions regarding the possible seasonal fluctuations.

The iodine content of the dry milk representative of the Winter and Summer production has been determined for the product made in each of the above localities. Composite samples were

prepared by selecting 12 ounces of the desiccated product representative of the day's production for each of 4 days on the same date of each month for the period from May to September inclusive as representing Summer production, and of the period from November to March as representing Winter production. The results from these analyses follow: The powder from the New York territory, Summer production, contained 145 parts of iodine per billion; the Winter production from the same territory contained 67 parts per billion. The powder from the Wisconsin territory, Summer production, contained 704 parts iodine per billion; the Winter production from the same territory contained 961 parts per billion.

SUMMARY

Analytical data are submitted showing the natural fluctuations in certain inorganic constituents of dry milk as affected by seasonal and territorial conditions in the States of New York and Wisconsin.

PROCEEDINGS OF THE TWENTY-FIFTH ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The Twenty-Fifth Annual Meeting of the American Dairy Science Association was held at Iowa State College, June 24, 25, and 26. There were approximately 250 in attendance. The industry was represented by men from all sections of the United States interested in the science and business of dairying. Members came from each of the three Pacific Coast States, from New England, from Middle Atlantic States, from Florida, Louisiana, South Carolina and other Southern States, and, of course, the North Central Section was especially well represented.

The program was a full one. The diversity of interests and activities of the dairy industry result in programs run more or less like a three ring circus. With meetings proceeding simultaneously on the economic phases of the industry, the manufacturing or product developments, and the production problems, one has difficulty in hearing all the papers in which he is interested.

There is an angle to these programs that seems somewhat unfortunate. You hear a paper read in which you are interested, one that you would like to go over carefully and digest thoroughly. But unlike the proceedings of some scientific societies, it has been the practice of the American Dairy Science Association not to publish the papers presented. Some of the reasons for this are: that many of the papers are merely a report of progress of investigations that will be published later in more detail; that some people do not want a paper published that will have to be briefed to the extent necessary for presentation in the time allowed on the program; then too, publication would perhaps mean the necessity of a review of the papers by the Program Committee prior to the meeting.

Those in attendance were enthusiastic in their praise of Professor Mortensen and his associates at Ames in the completeness of their arrangement for the comfort and amusement of the visi-

tors. The two banquets and a luncheon at Ames were greatly enjoyed. The dinner served on the lawn in the court of the splendid new Dairy Industry building at Ames, had such an attractive setting, that it will be long remembered by those who were so fortunate as to be present.

On the afternoon of the 26th the members were the guests of the Meredith Publishing Company, at Des Moines. They were conducted through the very modern and complete plant in which "Successful Farming" is published. Later we were taken to the Meredith farm near Des Moines, and shown their herd of registered Jerseys and served a delightful picnic supper. Mr. E. M. Harmon was in charge of ceremonies for the Meredith Publishing Company. On the following day the members were guests of the Cherry-Burrell Company and the Quaker Oats Company at Cedar Rapids. They were conducted through these large manufacturing plants and entertained at a dinner in the evening. Mr. J. A. McLean was in charge of the arrangements of this excursion for the Cherry-Burrell and Quaker Oats Companies.

At a business meeting of the association a committee for nomination of officers for 1931, consisting of Professors C. H. Eckles, R. B. Stoltz, and C. L. Roadhouse, submitted the following report:

For president:
Dr. H. A. Ruehe, University of Illinois.
Dr. H. B. Ellenberger, University of Vermont.
For vice-president:
Prof. C. E. Wylie, University of Tennessee.
Mr. F. W. Bouska, Beatrice Creamery Co., Chicago, Illinois.

The nominating committee calls attention to the provision of the constitution which makes possible additional nominations previous to November first by petition signed by not fewer than five members.

The following resolutions were presented and adopted.

WHEREAS the American Dairy Science Association has accepted the invitation of the Iowa State College, extended through the Dairy Industry Department, to hold its summer meeting of 1930 at the College; and

WHEREAS the Iowa Butter Manufacturers Association,
the Iowa Butter Makers Association,
the Iowa Ice Cream Manufacturers Association,
the Iowa Creamery Secretaries and Managers Association,
the Iowa State Brand Creameries Incorporated,
the Iowa State College Dairy Club,
the Meredith Publishing Company,
the Quaker Oats Company,
Cherry-Burrell Company and
Des Moines Milk Marketing Association,
have joined with the Faculty and Staff of the Dairy Industry Department and other departments of the college and the management of the Memorial Union, all under the leadership of Professor Mortensen in making our stay here most pleasant, comfortable, and informative.

Therefore, Be It Resolved by the American Dairy Science Association in convention assembled, that we tender to each individual and to each organization contributing to this splendid meeting and to the hospitality extended to our families and to ourselves—the thanks and appreciation of the members of the American Dairy Science Association individually and collectively.

WHEREAS the essential value of these meetings is dependent on the program arranged for, and the fulfilment of that program; and on the efficient conduct of the various meetings as well as on the work of the intervening year.

Therefore Be It Resolved that we hereby acknowledge our indebtedness to, and our appreciation of the excellent work of the program committee, the speakers, the officers, the editor of The Journal of Dairy Science, all of whom have contributed so much to that exchange of knowledge which makes for the progress of our science and industry and the inspiration of our members.

H. E. VAN NORMAN
A. A. BORLAND
Committee on Resolutions.

President Sherman discussed the desirability of some revisions in the constitution and by-laws of the association and asked Dr. C. H. Eckles to serve as Chairman of a committee to study and make recommendations for such revision. Dr. Eckles is to select his own committee.

There was some discussion as to whether the Divisions of the Association should be refunded fifty cents out or each membership fee, to defray costs of their annual meetings. The final action was that the parent association would continue to contribute up to twenty-five dollars a year toward the expenses of each Division pending recommendations of the Committee on Constitution.

Dr. L. A. Rogers moved that the Committee of the Association

appointed to act as an advisory committee with the National Research Council, be abolished. The motion was adopted.

Professor A. C. Ragsdale called attention to the National Dairy Improvement Contest sponsored by the St. Louis Chamber of Commerce, the outline of which follows:

NATIONAL DAIRY IMPROVEMENT CONTEST

Object: To promote better dairy management. The St. Louis Chamber of Commerce is interested in a National Dairy Improvement Contest on the following or a similar basis.

Unit: Considering the county as the unit and the county agent as the representative eligible to compete, the dairy extension departments of the various states would determine the winner from their state.

State Winners: The colleges would send in their winning report to be judged by a committee of competent judges.

Regional and National Winners: The judges would determine the regional winners, and from them select the sweepstakes winner.

Regions: There would be five regions—New England, Central, Central West, Southern, and Western.

Prizes: The winners would receive free trips to the National Dairy Exposition in St. Louis. They would also receive medals and certificates.

Basis of Awards: The awards would be made on the following basis:

	per cent
1. Analysis of problem	30
2. Methods used	30
3. Results	40

Work along the following lines would be important—Dairy Herd Improvement Associations, Calf Clubs, Bull Associations, Feeding Work, Pastures, Dairy Management, and all things tending to promote dairying. The coöperation of civic groups on this program would add to its effectiveness.

Control of Contest: Such a contest should be conducted under the supervision of the American Dairy Science Association and the U. S. Bureau of Dairy Industry.

The St. Louis Chamber of Commerce would provide the trips for the regional winners. The medals and certificates would be provided by the National Dairy Association in the name of the American Dairy Science Association.

Professor Ragsdale moved:

"That a special committee, with power to act, be appointed to develop plans and methods and supervise the conduct of a National Dairy Development Contest with special awards to be made at the time of the 1931 and succeeding National Dairy Shows. This committee to coöperate with a representative of the St. Louis Chamber of Commerce and the National Dairy Association, in the furthering of this contest."

This motion was adopted and President Sherman appointed the following committee:

R. W. Balderson, National Dairy Council, chairman
A. C. Ragsdale, University of Missouri, secretary
E. S. Savage, Cornell University
J. H. McClain, U. S. Bureau of Dairy Industry
W. M. Regan, University of California
C. S. Rhode, University of Illinois
E. M. Harmon, Meredith Publishing Company

Attention was called to the fact that many States have very small representations in the membership of the American Dairy Science Association. Some twenty-five of the States have fewer than five members. Those States having the greater number of members are:

New York	45	Michigan	16
California	38	Missouri	16
Illinois	32	District of Columbia	15
Ohio	21	Indiana	14
Wisconsin	17	New Jersey	12
Minnesota	17	Massachusetts	11
Pennsylvania	17	Iowa	11
		Vermont	10

Dr. Roadhouse of California told how he had written to men in commercial work in his State who were interested in the scientific aspects of the dairy industry, and explained to them the desirability of their being members of the American Dairy Science Association and getting The Journal of Dairy Science. He had a very favorable response and secured a number of new members. Dr. Eckles said he had been successful in securing new members in Minnesota in much the same way.

The executive committee voted in favor of holding the 1931 Annual Meeting on the Pacific Coast.

The Pacific Coast members had already arranged a tentative plan and schedule for the meeting and tour and had printed copies for distribution at Ames. According to these plans the meeting would be held on July 1 and 2 at Davis, California. Two different pre-convention trips are planned commencing

June 26 and continuing up to the time of the program to be followed by another tour lasting until July 16. There can be no doubt that the Pacific coast meeting will be outstanding as a combination of the program with sight-seeing and educational tours.

The following program was presented:

GENERAL SESSION

June 24, 1930

President J. M. Sherman, presiding

Registration—Memorial Union.
Call to order—(Great Hall, Memorial Union).
Address of Welcome. R. M. Hughes, President, Iowa State College.
President's Address. J. M. Sherman, Cornell University.
Announcements.
The economic situation of the dairy industry. Roy C. Potts, Bureau Agricultural Economics, U. S. D. A.
Significance and use of body measurements of dairy cattle.—W. W. Swett, Bureau of Dairy Industry, U. S. D. A.
The relation of plant operation to flavors in milk. H. A. Ruehe and P. H. Tracy, University of Illinois.
The mineral composition of bones of dairy cattle as influenced by age and nutrition. L. S. Palmer, University of Minnesota, and W. N. Neal, University of Florida.
Some factors affecting the development of nutritional anemia in the white rat. W. B. Nevens, University of Illinois.
Viability of Lacto-bacillus Acidophilis at different temperatures. M. J. Prucha, University of Illinois.
Packaging cheese without processing. L. A. Rogers, Bureau of Dairy Industry, U. S. D. A.
The nervous reaction of animals on a deficiency diet (Film). J. S. Hughes and H. W. Cave, Kansas State College.

DAIRY MANUFACTURERS AND BACTERIOLOGY

C. L. Roadhouse, Chairman, Section Dairy Manufactures, presiding

Studies in the use of chemical disinfectants—A. C. Fay, Kansas State College
A study of churn contamination. Part I. The growth of microörganisms on the wood of the churn. M. S. Libbert, University of Arkansas.
The importance of solubility of milk powders. J. C. Marquardt, N. Y. State Experiment Station.
The solubility of copper in milk under various conditions and its estimation by an electrochemical method. H. T. Gebhardt and H. H. Sommer, University of Wisconsin.
A comparison of the influence of iodized milk and of potassium iodide administered directly on the size and iodine content of the thyroid gland of rats. W. E. Krauss and C. F. Monroe, Ohio Experiment Station.

The chemical nature of the fatty materials in buttermilk. E. W. Bird, Iowa State College.

Studies in the manufacture of sweet-curd or baker's cheese. L. M. Thurston, University of West Virginia.

Modified Babcock tests for fat in ice cream. L. K. Crowe, University of Nebraska.

Some observations on the basic viscosity of ice cream mixes. J. C. Hening, N. Y. State Exp. Station.

Influence of the source of fat and serus solids on overrun and quality in ice cream. J. M. Jensen, Michigan Agricultural College.

A visit to the Dairy Industry Building and the Dairy Farm.

Mixer at the Dairy Industry Building.

Dairy Production and Official Testing

H. B. Ellenberger, Chairman, Section of Dairy Production, presiding

Influence of water bowls on water consumption of dairy cows. C. Y. Cannon, Iowa State College.

Influence of sunshine on the growth and on the milk of dairy heifers. T. M. Olson, South Dakota Agricultural College.

Effect of delayed milking and pressure on milk secretions. W. E. Petersen and T. Rigor, University of Minnesota.

The effects of storage on the composition of root crops. K. S. Morrow, University of West Virginia.

Results of recent tabulations of dairy herd improvement records. J. C. McDowell, Bureau of Dairy Industry, U. S. D. A.

The therapeutic effect of cod liver oil and ultra-violet light upon the nutrition of calves. S. E. Bechdel, Pennsylvania State College.

Energy and nitrogen metabolism of dairy calves. Samuel Brody, University of Missouri.

A study of the comparative efficiency of electrically operated cooling tanks versus ice in the cooling of milk. J. H. Frandsen, Massachusetts Agricultural College.

The effect of chemical sterilizers on the rubber parts of milking machines. E. H. Parfitt, Purdue University.

Vitamin A as a limiting factor in the growth of dairy calves that receive no roughage. W. M. Regan, University of California.

A visit to the Dairy Industry Building and the Dairy Farm.

Mixer at the Dairy Industry Building.

June 25

Dairy Products and Bacteriology

C. L. Roadhouse, Chairman, Section of Dairy Manufactures, presiding

A test for fat in condensed and evaporated milk and ice cream. W. D. Swope, Pennsylvania State College.

Relation of temperature in ice cream to the distribution of certain constituents in its solid and liquid phases. V. C. Cole, University of California.

The effect of dilution on the titratable acidity of cows' milk. Jules Menos and H. H. Sommer, University of Wisconsin.

Quantitative changes in the microflora during the manufacture of butter. S. T. Coulter, University of Minnesota.

Relation of feathering and heat stability of cream to fat clumping due to homogenization. F. J. Doan, Pennsylvania State College.

The relation between the age of cream when delivered at the buying station and the quality of the resulting butter. V. C. Manhart, Purdue University.

Surface taint in butter. H. A. Derby and B. W. Hammer, Iowa State College.

The electric charges on milk fat globules and their relation to various dairy phenomena. G. C. North and H. H. Sommer, University of Wisconsin.

Section meeting, committee reports, business.

Dairy Production and Official Testing

H. B. Ellensberger, Chairman, Section of Dairy Production, presiding

Cottonseed meal as the sole concentrate for dairy heifers. Earl Weaver, Oklahoma Agricultural College.

The individuality of the four quarters of the udder. C. W. Turner, University of Missouri.

The comparative feeding value of immature versus mature soybean hay for milk and fat production. J. H. Hilton, Purdue University.

The contribution of the Bowlker Hybrid herd to our knowledge of dairy cattle breeding. W. W. Yapp, University of Illinois.

A study of the sugar content of the blood of dairy animals. Ralph Hodgson and W. H. Riddell, J. S. Hughes, Kansas State College.

Preparation and methods of analysis of bones of dairy cattle. W. N. Neall, University of Florida, and L. S. Palmer, University of Minnesota.

Value of grinding roughage. L. H. Fairchild, Letz Manufacturing Company.

The number of daughters necessary to prove a sire. Jay L. Lush, Iowa State College.

Section meeting, committee reports, business.

Extension Section

G. A. Williams, Chairman, Dairy Extension Section, presiding

Improving the quality of dairy products. J. B. Parker, Bureau of Dairy Industry, U. S. D. A.

Comparison of unit requirements for milk production in northeastern, central, and southwestern districts. C. H. Willoughby, University of Florida.

The value of testers' conferences.

Discussion led by A. C. Baltzer, Michigan State College.
E. N. Shultz, Iowa State College.
Ed. Hansen, University of Minnesota.
C. R. Gearhart, Pennsylvania State College.

Feeding Schools.

Discussion led by H. R. Searles, University of Minnesota.
Ivan McKellip, Ohio State University.
E. A. Gannon, Purdue University.

Demonstration of a mechanical device for rapid calculations in balancing rations. C. M. Long, Blue Valley Creamery Company.

General Program

H. C. Jackson, Vice-President, presiding

The protein problem in dairy feeding. W. J. Fraser, University of Illinois.

Determination of the hemoglobin content of the blood of dairy animals. H. J. Brooks and J. S. Hughes, Kansas Agricultural College.

Vitamin A content in the milk of different breeds. I. L. Hathaway, H. P. Davis, University of Nebraska.

An experimental review of Tillmann's method for detecting the neutralization of milk and cream. Kenneth Week and H. H. Sommer, University of Wisconsin.

A method for improving the quality of butter of a state without excessive expenditures. L. C. Thomsen, University of Wisconsin.

The use of cocoa, chocolate liquor, and prepared chocolate syrups in the manufacture of chocolate ice cream. W. J. Caulfield, Kansas State College.

Some bacteriological and temperature studies in milk plants. C. W. Leete, Bureau of Dairy Industry, U. S. D. A.

The use of chemical deodorants in cream for buttermaking. W. H. Martin and C. L. Smith, Kansas State College.

The effect of uneven intervals of milking upon the lactose and chloride content of milk. C. L. Roadhouse and J. L. Henderson, Univerity of California.

Speed of dasher and scraper as affecting the quality of ice cream. E. L. Reichart, University of Nebraska.

Development of butter culture. R. Farmer and B. W. Hammer, Iowa State College.

Business meetings of sections.

Extension Section

June 26

G. A. Williams, Chairman, Dairy Extension Section, presiding

Standardized rules for Official Testing of all dairy breeds. E. L. Anthony, Michigan State College.

The data necessary to prove a purebred sire. Warren Gifford, University of Missouri.

The use of herd improvement records as a means of proving dairy bulls. J. C. McDowell, Bureau of Dairy Industry, U. S. D. A.

Locating and retaining proved sires.

Discussion led by J. A. Simms, Connecticut Agricultural College.
E. N. Shultz, Iowa State College.
E. J. Perry, Rutgers College.
E. T. Wallace, Purdue University.
M. J. Regan, University of Missouri.

Dairy Products and Bacteriology

J. M. Sherman, President American Dairy Science Association, presiding

Yeast and mold content of strawberry ice cream. P. A. Downs, University of Nebraska.

Correlation between the annual butterfat production and the feed cost of dairy cows. C. W. McIntyre.

The effect of feeding potatoes to dairy cows on the production and quality of milk and butterfat. J. R. Dice, North Dakota Agricultural College.

An organism rapidly increasing the acid number of butter. R. V. Hussong and B. W. Hammer, Iowa State College.

The effects of rice by-products upon the fatty acid of butterfat. M. S. Libbert and H. E. Dvorachek, University of Arkansas.

The relation between the per cent of fat in the cream and the fat loss in the buttermilk. E. W. Bird and M. Mortensen, Iowa State College.

Sweetened frozen cream for the manufacture of ice cream. Walter V. Price, University of Wisconsin.

Properties of casein for paper coating. R. W. Bell, Bureau of Dairy Industry, U. S. D. A.

The transition points of some milk fats. G. A. Richardson, University of California.

The precipitation of gelatin in chocolate ice cream. Floyd E. Kurtz, Bureau of Dairy Industry, U. S. D. A.

Influence of the acidity of the butter culture on the quality of butter. Chris Jensen, North Dakota Agricultural College.

Observations on churn sanitation. Henry Morrison, University of Minnesota.

College Instruction Program

E. L. Anthony, Michigan Agricultural College, presiding

Do we need a dairy education. A. D. Burke, Oklahoma Agricultural College.

College Dairy Herds, size, number of breeds, utilization. Earl Weaver, Oklahoma Agricultural College.

How much time should be given to teaching dairy cattle judging. W. E. Petersen, University of Minnesota.

Inspection and judging trips, value, time allowed. J. R. Dice, North Dakota Agricultural College.

How essential is laboratory work and how much time should it take. A. A. Borland, Pennsylvania State College.

Can good manufacturing instruction be given without the operation of a college creamery. E. L. Reichart, University of Nebraska.

Maintaining the student interest. Elmer Hansen, Iowa State College.

Teaching dairy feeds by the use of problems. R. B. Becker, University of Florida.

Lecture, recitation, or problem. L. K. Crowe, University of Nebraska.

Is there a place for a market milk course. H. Macy, University of Minnesota.

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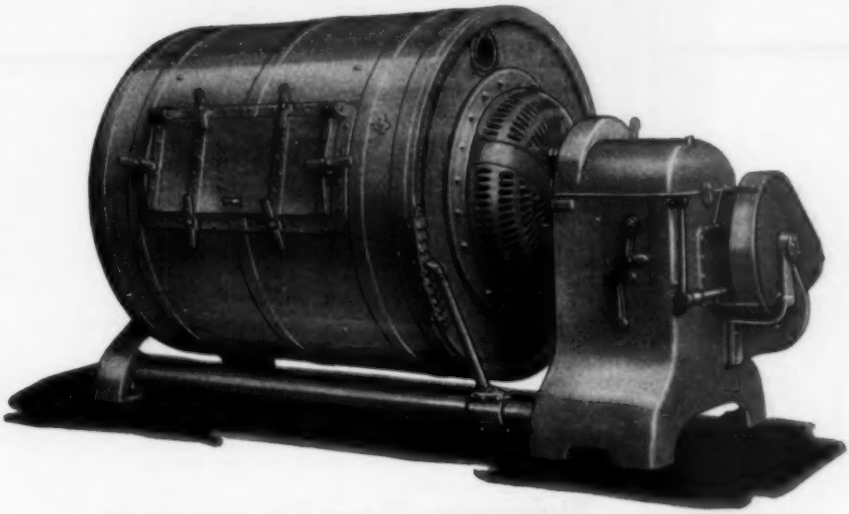

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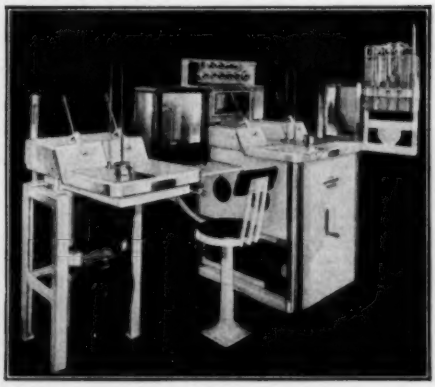

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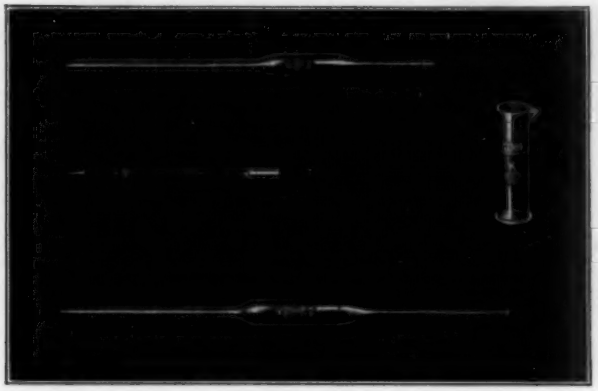

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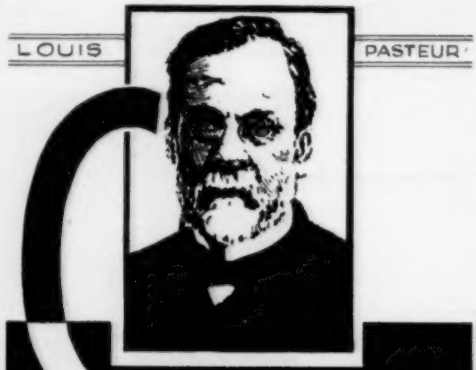

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